ON REACTION RATE FOR HYDROLYSIS OF SUBSTITUTED CYCLIC NITROSOUREAS [1]

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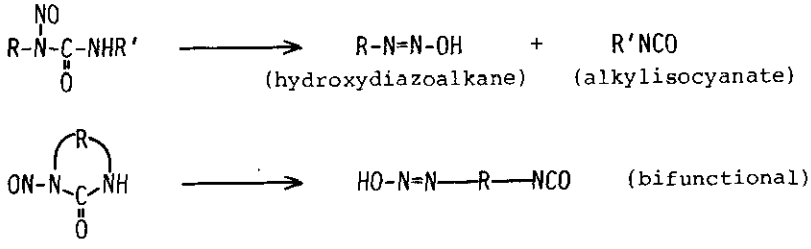
The rates for hydrolyses of 20 derivatives of N-nitroso-dimethyleneurea and N-nitrosotrimethyleneurea were measured. Discussion is made on the relationship between structure and rate for hydrolysis.

Many N-nitrosoalkylureas are known to be carcinogenic and/or mutagenic [2] and some are clinically used as anti-cancer agent.[3] These biological activities all are considered to stem mainly from their alkylating ability and possibly to be related to their carbamoylating ability.[4] Thus, this class of compounds undergo hydrolysis to split into two fragments even in a physiological condition; a hydroxydiazoalkane which is an alkylating agent and an alkylisocyanate which is a carbamoylating agent.[4,5] Alkylations of DNA may cause gene-lesion leading to carcinogenesis, mutagenesis, and cell-inactivation, and carbamoylations of enzyme proteins including those for the DNA repair may produce inhibitory effects on the enzymic repairs of the gene-lesion induced with the alkylating moiety of nitrosoureas.[6] In order to increase in the cell-inactivating efficiency of nitrosoureas, intending to improve chemotherapeutic index of anti-cancer nitrosoureas, our attention has been focused on cyclic nitrosoureas which undergo hydrolysis to form a bifunctional intermediate involving both an alkylating and a carbamoylating centers in the molecule.



As one of our serial studies along this line, this paper presents the rates of hydrolyses of 20 derivatives of N-nitrosodimethyleneurea and N-nitrosotrimethyleneurea. Discussion will be made on the substituent effect on the rate of hydrolysis which is considered to be the obligatory step for the biological activity of these nitrosoureas.

The compounds used, which were synthesized by T. Mizoguchi and his coworkers of Tanabe Pharmaceutical Co. Ltd., [7] are 4-substituted N^1-nitrosodimethyleneurea, 4-substituted N^1-nitrosotrimethyleneurea, and 5-substituted N^1-nitrosotrimethyleneurea shown in Table I. The rate of hydrolysis was obtained by treating a nitrosourea to be examined in 1.77% phosphate buffer (pH 7.0) at 37°, followed by tracing the optical density at about 400 nm of the solution with a Shimazu-UV-210A uv-spectrometer. The concentration was about 1 mg/ml of the solvent. In Table I are shown the pseudo-first order rate constants (k_{obs}) and the half-lives ($t_{1/2}$) of cyclic nitrosoureas, including methyl- and ethyl-nitrosourea for comparison.

Dependence on Ring Size

5-Memebered nitrosodimethyleneurea (I) was much more readily hydrolyzed than 6-membered nitrosotrimethyleneurea (VIII). This may be due to release from the ring strain involved in cyclic starting materials by the ring opening at the transition state. It is worth noting that this trend is reverse to those found in the alkaline hydrolyses of 5- and 6-membered lactones [8] and lactams [9], where an attack of OH^- to the C=O carbon is involved in the rate-determining step.

Substituent Effect

Nitrosodimethyleneureas The rates of 4-substituted N^1-nitrosodimethyleneureas become smaller in the following order of the substituent:

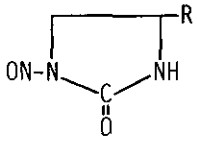
$$H > -CH_2OH > -CH_2Cl > -CH_2OMs$$
$$H > -CH_2CH_2OH > -CH_2CH_2Cl > -CH_2CH_2OMs$$

Another feature of the structure-rate relationship is that replacement of a methyl substituent with the corresponding ethyl substituent makes the rate larger in all the cases examined, i.e., $-CH_2X < -CH_2CH_2X$, where X is either of -OH, -Cl, or -OMs.

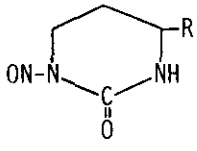
Nitrosotrimethyleneureas In striking contrast, regardless of the position of the substituent, 4- or 5-position, the rates become larger, reversely to those of the dimethylene derivatives, in the following order of the substituent.

Table I. Pseudo-First Order Rate Constants and Half-Lives for Hydrolysis of Nitrosoureas in Phosphate Buffer (pH 7.0) at 37°

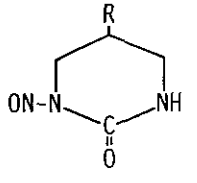
Substituent	Compound No.	k_{obs} ($10^{-5}sec^{-1}$)	$t_{1/2}$ (min)
4-substituted N-nitrosodimethyleneureas			
-H	I	210.0	5.5
$-CH_2OH$	II	97.4	12.2
$-CH_2Cl$	III	27.6	41.8
$-CH_2OMs$	IV	17.1	67.4
$-CH_2CH_2OH$	V	123.0	9.4
$-CH_2CH_2Cl$	VI	113.0	10.2
$-CH_2CH_2OMs$	VII	81.3	14.2
4-substituted N-nitrosotrimethyleneureas			
-H	VIII	8.37	138.0
$-CH_2OH$	IX	31.4	36.8
$-CH_2Cl$	X	54.2	21.3
$-CH_2OMs$	XI	93.2	12.4
$-CH_2CH_2OH$	XII	16.0	72.2
$-CH_2CH_2Cl$	XIII	25.6	45.2
$-CH_2CH_2OMs$	XIV	37.0	31.2
5-substituted N-nitrosotrimethyleneureas			
-H	VIII	8.37	138.0
$-CH_2OH$	XV	17.5	66.0
$-CH_2Cl$	XVI	40.7	28.4
$-CH_2OMs$	XVII	54.0	21.4
$-CH_2CH_2OH$	XVIII	9.24	125.0
$-CH_2CH_2Cl$	XIX	12.3	94.0
$-CH_2CH_2OMs$	XX	14.0	82.5
Acyclic N-nitrosoalkylurea			
N-nitrosomethylurea	XXI	49.8	23.2
N-nitrosoethylurea	XXII	54.2	21.3



4-substituted
N-nitrosodimethyleneureas



4-substituted
N-nitrosotrimethyleneureas



5-substituted
N-nitrosotrimethyleneureas

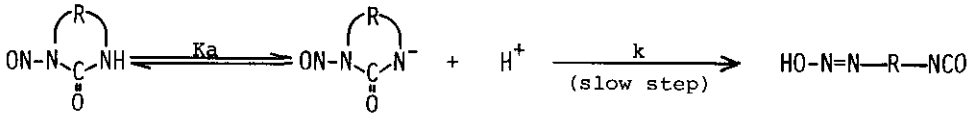
$$H < -CH_2OH < -CH_2Cl < -CH_2OMs$$

$$H < -CH_2CH_2OH < -CH_2CH_2Cl < -CH_2CH_2OMs$$

The rate for a methyl substituted derivative is always larger than the corresponding ethyl derivative, i.e., $-CH_2X > -CH_2CH_2X$. It is further revealed that a 4-substituted derivatives is more readily hydrolyzed than the corresponding 5-substituted one in all the cases examined.

Dependence of the Rate on PH

The rate of hydrolysis is linearly proportional to the concentration of OH^- in near neutral media examined, as shown in Fig. 1. The reaction mechanism was already proposed by several authers as follows.[10]



Therefore, the rate can be expressed by eq. 1.

$$\text{Rate} = k \frac{Ka}{Ka + [H_3O^+]} [Nu] \quad \text{-------- eq. 1}$$

where Ka is the dissociation constant, k is the rate constant, and [Nu] is the sum of the concentrations of nitrosourea and its deprotonated conjugate base.

In case of $Ka << [H_3O^+]$,

$$\text{Rate} = k\ Ka\ [Nu]/[H_3O^+]$$

$$= k\ (Ka/Kw)\ [OH^-]\ [Nu] \quad \text{------ eq. 2}$$

and in case of $Ka >> [H_3O^+]$,

$$\text{Rate} = k\ [Nu]$$

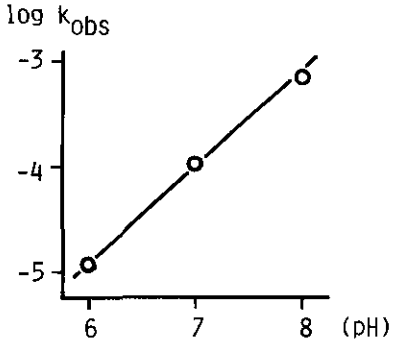


Fig. 1.
log k_{obs} plots versus pH

Since the rate was proportional to $[OH^-]$, the reaction is regarded to be kinetically expressed by eq. 2 under the conditions employed.

Hence, $\log k = \log k_{obs} - pH + pKa$.

Kinetic Parameters for Hydrolysis

The rates of some of the derivatives were measured at different temperatures for evaluation of the energy of activation (Ea) and the frequency factor (log A) or the entropy of activation ($\Delta S^{\ddagger}$), the results being summarized in Table II. It is of interest that $\Delta S^{\ddagger}$(obs), which was calculated with k_{obs}, is possitive in each case examined; hence $\Delta S^{\ddagger}$ is also possitive because k is larger than k_{obs} in these cases. This supports that the rate-determining step involves the ring opening of conjugate

Table II. Kinetic Parameters for Hydrolysis of Nitrosoureas in Phosphate Buffer (pH 7.0)

Compound	Temp.	k_{obs} ($10^{-5}sec^{-1}$)	Ea(Kcal/mol)	log A(obs)	$\Delta S^{\ddagger}$(obs)(e.u.)
X	25°	7.97	28.1	16.6	17.4
	37°	50.0			17.3
XIII	25°	3.64	28.5	16.5	17.0
	37°	23.2			16.9
XIX	25°	2.99	28.1	16.1	15.1
	37°	19.2			15.1
VIII	30°	3.33	27.6	15.4	12.0
	40°	14.4			12.0
XXI(acyclic)	30°	15.2	27.4	15.9	14.2
	40°	64.7			14.1

base of the nitrosourea in an unimolecular process, as been kinetically expressed by eq. 1, *although an alternative mechanism* proposed by Snyder and Stock, which involves an attack of OH^- to the C=O carbon in the rate-determining step, is not *completely eliminated.*[11]

Discussion

It seems that the entropy term is an important factor for determination of the rate; the order of the relative rates is same as that of the magnitudes of entropy, as far as the cyclic derivatives shown in Table II are concerned;

X > XIII > XIX > VIII

However, the substituent effect found in the present study seems to be hardly explained in simple terms, such as steric hindrance, inductive electronic effect, etc. More data of the kinetics of all the compounds used here and some related classes of compounds seem to help to correlate the rate of hydrolysis with the chemical structure of compounds. In addition to studies along this line, biological activities including mutagenic and carcinostatic activities of these cyclic nitrosoureas are now under investigation in our laboratory.

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REFERENCES

1 This work constitutes Part XIX of a series entitled "Studies on Chemical Carcinogens". Part XVIII: O. Ogawa, Y. Kawazoe, and H. Sawanishi, Chem. Pharm. Bull. Tokyo, in press.

2 Y. Seino, M. Nagao, T. Yahagi, A. Hoshi, T. Kawachi, and T. Sugimura, Cancer Res. 1978, 38, 2148. R. B. Brundrett, M. Colvin, E. H. White, J. McKee, P. E. Hartman, and D. L. Brown, ibid., 1979, 39, 1328. K. Yano and M. Isobe, ibid., 1979, 39, 5147.

3 T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, J. Med. Chem., 1963, 6, 669. J. A. Montgomery and J. G. Mayo, ibid., 1974, 17, 477. T. P. Johnston, G. S. McCaleb, S. D. Clayton, J. L. Frye, C. A. Krauth, and J. A. Montgomery, ibid., 1977, 20, 279. J. A. Montgomery, G. S. McCaleb, T. P. Johnston, J. G. Mayo, and W. R. Laster, Jr., ibid., 1977, 20, 291.

4 G. P. Wheeler, B. J. Bowdon, J. A. Grimsley, and H. H. Lloyd, Cancer Res., 1974, 34, 194. M. Colvin, R. B. Brundrett, W. Cowens, I, Jardine, and D. B. Ludlum, Biochem. Pharmacol., 1976, 25, 695. L. C. Panasci, P. A. Fox, and P. S. Schein, Cancer Res., 1977, 37, 3321. H. E. Kann, Jr., ibid., 1978, 38, 2363. J. M. Heal, P. A. Fox, D. Doukas, and P. S. Schein, ibid., 1978, 38, 1070.

5 A. Begleiter, H. P. Lam, and G. J. Goldenberg, Cancer Res., 1977, 37, 1022. J. W. Lown, L. W. McLanghlin, and J. A. Plambeck, Biochem. Pharmacol., 1979, 28, 2115. D. J. Reed, H. E. May, R. B. Boose, K. M. Gregory, and M. A. Beilstein, Cancer Res., 1975, 35, 568.

6 A. J. Fornace, Jr., K. W. Kohn, and H. E. Kann, Jr., Cancer Res., 1978, 38, 1064. J. M. Heal, P. A. Fox, and P. S. Schein, ibid., 1979, 39, 82. H. E. Kann, Jr., B. A. Blumenstein, A. Petkas, and M. A. Schott, ibid., 1980, 40, 771. And the references cited therein.

7 T. Mizoguchi, Chem. Pharm. Bull. Tokyo, to be published.

8 R. Huisgen and H. Ott, Tetrahedron, 1959, 6, 253.

9 T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanism" (Benjamin, Inc., New York), 1966, vol. 1, p.22.

10 E. R. Garrett and S. Goto, Chem. Pharm. Bull. Tokyo, 1973, 21, 1811. And the references cited therein.

11 J. K. Snyder and L. M. Stock, J. Org. Chem., 1980, 45, 1990.

Added in proof. Fig. 1 shows the plots of log k_{obs} of N-nitrosotrimethyleneurea.

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