[Chem. Pharm. Bull. 21(8)1811—1823(1973)] UDC 547.495.4.04 : 542.938

Kinetics of Solvolyses of Various N,N'-Dialkyl-N-nitrosoureas in Neutral and Alkaline Solutions[1)]

EDWARD R.GARRETT[2)] and SHIGERU GOTO[2a)]

The Beehive, College of Pharmacy, University of Florida[2)]

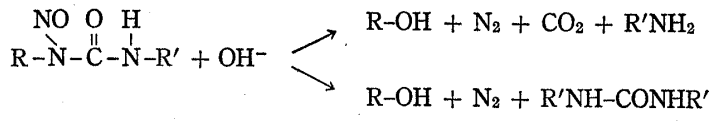
(Received March 1, 1973)

a) The neutral and alkaline solvolyses of N-methyl-N'-*p*-fluorophenyl-N-nitrosourea, 1,6-dimethyl-1,6-dinitrosobiurea, N-methyl-N'-phenethyl-N-nitrosourea, trimethylenebis-N-methyl-N-nitrosourea and N,N'-dimethyl-N-nitrosourea are subject to spontaneous and specific hydroxide ion catalysis, *i.e.* k_{app} (sec^{-1}) $= k_0 + k_{OH}[OH^-]$. The decreasing order of reactivity is in the order given and is consistent with the fact that electron-withdrawing substituents abet hydroxyl ion attack by inducing a more positive center. The fact that substitution of an alkyl for a hydrogen on the N'-nitrogen inhibits hydrolysis favors the adjacent carbonyl carbon as the primary focus of hydroxide ion attack.

b) The log *k*-pH profiles of N,N'-dimethyl-N-nitrosourea and 1,6-dimethyl-1,6-dinitrosobiurea show a deviation in the linearity of the alkaline branch which can be attributed to a kinetic p*K*a' where these compounds may exist as anions at higher pH values and thus be more resistant to hydroxide ion attack.

c) The gas chromatographically analyzed alcohol products of alkaline degradation of N-alkyl-N'-nitrosoureas are various in several cases and thus clearly implicate a potentially rearranging carbonium ion intermediate in the alkaline solvolyses of these compounds. The N-*n*-butyl compound gives a mixture of *n*-butyl alcohol in 45% yield, and secondary butyl alcohol in 24% yield for pH values greater than 7.0. The fact that the N-isobutyl compound yields *t*-butyl alcohol (76%), *sec*-butyl alcohol (22%) and isobutyl alcohol (18%) also implicates a methyl shift. The N-alkyl- and N-benzyl- compounds yield only the corresponding alcohols.

The N-nitrosoureas are active as anticancer agents,[3–7)] carcinogens,[8)] and mutagens.[9)] They demonstrate a high instability in aqueous solutions, even at neutral pH values, with decomposition rates increasing with pH.[10–12)] The normal products of neutral and alkaline degradation of 1-substituted and 1,3-disubstituted 1-nitrosoureas are presumed to include an alcohol and nitrogen.[10–12)] However, 1,3-bis (2-chloroethyl)-1-nitrosourea and several



1) Supported in part by Public Health Service Grant No. GM-09864, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland, U.S.A.

2) Location: *Gainesville, Florida 32601, U.S.A.*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Okayama University, Tsushima Okayama 700, Japan.*

3) W.H. Skinner, H.F. Gram, M.O. Greenberg, and B.R. Baker, *J. Med. Pharm. Chem.*, **2**, 299 (1960).
4) K.A. Hyde, E. Acton, W.A. Skinner, L. Goodman, J. Greenberg, and B.R. Baker, *J. Med. Pharm. Chem.*, **5**, 1 (1962).
5) T.P. Johnston, G.S. McCaleb, and J.A. Montgomery, *J. Med. Pharm. Chem.*, **6**, 669 (1963).
6) H.E. Skipper, F.M. Schabel, Jr., M.W. Trader, and J.R. Thomson, *Cancer Res.*, **21**, 1154 (1961).
7) F.M. Schabel, Jr., T.P. Johnston, G.S. McCaleb, J.A. Montgomery, W.R. Laster, and H.E. Skipper, *Cancer Res.*, **23**, 725 (1963).
8) H. Druckrey, R. Preussmann, D. Schmähl, and M. Muller, *Naturwissenshaften*, **48**, 165 (1961).
9) L. Pasternak, *Acta biol. med. Ger.*, **10**, 436 (1963).
10) E.R. Garrett, *J. Pharm. Sci.*, **49**, 767 (1960).
11) J.L. Boivin and P.A. Boivin, *Can. J. Chem.*, **29**, 478 (1951).
12) E.R. Garrett, S. Goto, and J.F. Stubbins, *J. Pharm. Sci.*, **54**, 119 (1965).

other similar chloroalkyl derivations in aqueous solution decomposed anomalously to produce acetaldehyde, HCl, N_2 and derivatives of 2-chloroethyl isocyanate. Fluorine analogs of these compounds decomposed in the normal manner.[13]

This paper considers the kinetics of solvolysis of additional 1,3 disubstituted nitrosoureas and the nature and yield of various alcohols from the alkaline solvolysis of several alkyl nitrosoureas.

Experimental

Kinetic Studies——The general procedure was to dissolve a specific N-methyl-N-nitroso-N′-substituted urea, I, in 100 ml distilled water maintained at the temperature of the prospective kinetic study. This original solution was subsequently diluted 1: 99 with the appropriate buffer solution which was also thermally preequilibrated. The difficultly solubilized N-methyl-N-nitroso-N′-phenethyl and N-methyl-N-nitroso-N′-fluorophenyl ureas were initially dissolved in a small amount of ethanol prior to their dissolution in water. In these cases, 5 ml of the nitrosourea solution was added to 90 ml of the appropriate thermally preequilibrated buffer solution and made up to a total volume of 100 ml. The alcohol concentration of the final reactant solution was 0.4% by volume. In general, the final concentration of N-nitrosourea, *ca.* 10^{-4}M, was optimum for reading spectrophotometric absorbances.

For the slow reactions, $t_{1/2}>30$ min, the aliquots were rapidly cooled to room temperature before reading at the appropriate wavelengths on the Beckman Model DU Ultraviolet (UV) spectrophotometer. The rates of fast reacting materials had to be obtained differently. The pertinent reacting solution was permitted to degrade in the thermostatted cuvette holder of the Beckman DU. The absorbance was continuously plotted on a time-calibrated Sargent SRL recorder with the aid of a Beckman energy recording adaptor (ERA). No photolytically catalyzed reactions were observed.

The conditions for the various studies and the composition of the various phosphate buffers are given in the various tables. When necessary, ionic strength was adjusted by the addition of potassium chloride. The wavelengths that were characteristic of the λ_{max} of the N-methyl-N-nitroso-N′-R′ substituted ureas, I, for various R′ were. H, 232 mμ; CH_3, 230 mμ; phenethyl, 230 mμ; *p*-fluorophenyl, 265 mμ. The λ_{max} for 1-1′-trimethylene bis[3-methyl-3-nitroso] urea was 225 mμ, and for 1,6-dimethyl-1,6-dinitrosobiurea was 245 mμ, and for tetrahydro 1,3-dinitroso-2(1H)-pyrimidinone was 260 mμ.

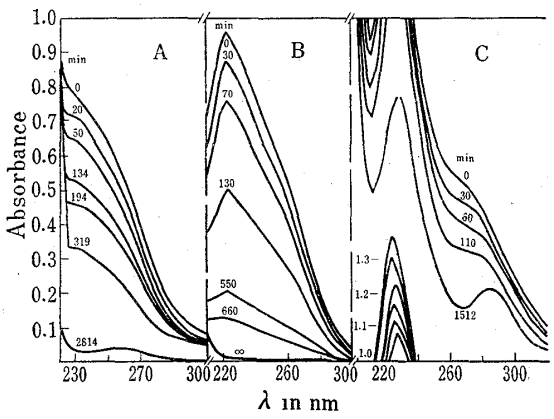


Fig. 1. Typical Spectrophotometric Curves as a Function of Time in Minutes for several N′-Substituted-N-methyl-N-nitrosoureas

A) N-methyl-N′-phenethyl-N-nitrosourea at pH 7.61 and 35.1°; B) N,N′-dimethyl-N-nitrosourea at pH 7.60 and 40.0°; and C) N-methyl-N′-*p*-fluorophenyl-N-nitrosourea at pH 6.42 and 30.0°

The absorbance at this λ_{max} was read as a function of time until it either disappeared or, as in the cases of the phenyl compounds, approached an asymptotic value. Typical spectrophotometric curves as a function of time are given in Fig. 1 for N-methyl-N′-phenethyl-N-nitrosourea, N,N′-dimethyl-N-nitrosourea and N-methyl-N′-*p*-fluorophenyl-N-nitrosourea and were recorded on a Cary UV recording spectrophotometer, Model 15.

No significant effects were observed on variation of buffer concentrations, substrate concentrations or ionic strength.

The nitrosoureas were obtained from the Southern Research Institute of Birmingham, Alabama through the courtesy of Dr. John A. Montgomery. Their structures and polarographic behavior have been given previously.[14]

Investigation of the Degradation Products of N-Nitrosoureas by Gas Chromatography——Amounts of N-alkyl-N-nitrosoureas sufficient to prepare 10 ml of a 10^{-2}M solution were weighed on a Mettler balance into a 10 ml injectable vial. A solution (10 ml) of 0.1M NaOH was added and the mixture allowed to stand overnight. The evolution of nitrogen was observed early in the reaction. The reaction mixtures were sealed with a crimped aluminum cap over a rubber stopper to prevent the escape of volatile products.

The following morning, a 5 ml sample was transferred to a new 10 ml injectable vial and 4.0 g of NaCl was added. Extraction was effected with 2 ml of diethyl ether by shaking a min on a Vortex mixer. After

13) J.A. Montgomery, R. James, G.S. McCaleb, and T.P. Johnston, *J. Med. Chem.*, **16**, 668 (1967).
14) E.R. Garrett and A.G. Cusimano, *J. Pharm. Sci.*, **55**, 702 (1966).

standing 5 min to separate the phases, 5 or 10 microliters of the ether extract with a dodecane internal standard was injected into an F. and M. Model 700 gas chromatograph equipped with either an electron capture or flame ionization module. Qualitative identification and quantification of products were effected by injecting a 10 microliter sample from an ether extract of 0.000, 0.001, 0.003, 0.005, 0.007 and 0.010M solutions (at the pH of degradation of the N-nitroso compound) of the predicted alcohols expected from the degradation. The electron capture detector was used to search for small quantities of intermediates in the reaction whereas the flame ionization detector was used for the major products.

The conditions used in the gas chromatography were: 4 foot stainless steel column packed with 20% carbowax, 20M on 60—80 mesh chromosorb W, detector temperature, flame 250,° electron capture 190°; injection port temperature, 160°; gas flow rates, He 60 ml/min at 30 psig, H_2 preset at 10 psig, Air preset at 30 psig, argon–methane (90: 10%) at 30 psig and 140 ml/min; oven temperature, 70° for *n*-butyl, isobutyl and allyl N-nitrosoureas, 145° for benzyl-N-nitrosourea. Other conditions were: range 100, attenuation 2, chartspeed at 4 inches/min and *e.c.* pulse 15.

Acetate derivatives of normal and secondary butyl alcohols were prepared from 1 ml of the alcohols and 1.5 ml of acetyl chloride. The excess acid was neutralized with conc. NaOH solution to a pH of 8. An aliquot (10.0 μl) of the oil phase of the acetylated alcohol was diluted with 2.0 μl of ether and 10 μl of the ether solution of the internal standard (dodecane) was added. Unfortunately, the retention times of the primary and secondary butyl alcohols were too close to those of the acetate derivatives to permit satisfactory resolution. The derivatization procedure was repeated with benzoyl chloride with a drop of pyridine for a catalyst. The sealed vials were heated at 70° for one hour, cooled and neutralized with concentrated NaOH. Two drops of the benzoyl alcohols were added to 10 ml of ether. Aliquots (2 μl) of the resultant solutions were analyzed chromatographically with the flame ionization detector with an oven temperature of 125°. Peaks assignable to the benzoate were clearly observed that were not assignable to the alcohols, pyridine or benzoyl chloride.

A series of solutions, 0.001 to 0.010M in the alcohol were prepared in 6.80 pH phosphate buffer and to each 40 ml aliquot, 14 g NaCl, 40 ml of ether and 20 μl of internal standard (dodecane) were added. The mixtures were shaken for 5 min and the ether phase separated and placed in separate vials. The ether was evaporated under ambient conditions. Benzoyl chloride (0.5 ml) was added to each vial and the vials sealed. The vials were heated for one hour at 90°, cooled and 10 ml of 0.6N Na_2CO_3 added. The resultant solutions were heated for 15 min at 90°. They became clear and were cooled.

Ether (4.0 ml) was added to each vial and the vials were shaken. The phases separated and 10 μl of ether extract was gas chromatographically analyzed. Alkaline degraded *n*-butyl-N-nitrosourea solutions were neutralized and subjected to the same extraction and derivatization procedures as was effected with the alcohols.

Calculation and Result

Rate Constants

The first-order rate constants, k, were calculated from the slopes of plots of the logarithm of the difference in absorbance, A, at time, t, and the final absorbance, A_∞, against time in accordance with

$$\log (A - A_\infty) = -kt/2.303 + \log A_0 \qquad (1)$$

Typical first-order plots for the solvolysis of these compounds are given for the typical example of N,N′-Dimethyl-N-nitrosourea at various pH values (Fig. 2).

Kinetics of N,N′-Dimethyl-N-Nitrosourea Degradation

The log k-pH profiles for the solvolysis of N,N′-dimethyl-N-nitrosourea are given in Fig. 3 as based on the data of Table I. Particular attention should be given the 35.0° data.

Above a pH of 9, specific hydroxyl ion catalyzed solvolysis is indicated by the fact that the log k *vs* pH plot approaches a slope of +1 in this region. Below this pH value, the rates of solvolysis appear to be affected by a) a pH independent solvolysis of rate constant k_0 characterized by a constancy of rate with pH below a pH value of 6.0 and b) a change in the nature of the hydroxyl ion catalyzed solvolyzing species at high or alkalinities in the pH range 7.5—9.0. Thus, in addition to the pH independent solvolysis below pH 6.0 there appears to be a concomitant attack of hydroxyl ion on the species present in acid solution and a lessened attack of hydroxyl ion on a modified species as evidenced by an inflection in the log k-pH

TABLE I. Conditions and Apparent First Order Rate Constants (k in sec^{-1}) for the Solvolysis of 10^{-4}M N,N′-Dimethyl-N-nitrosourea

°C	Buffer composition		pH	10^6k
	$[H_2PO_4^-]$	$[HPO_4^=]$		
30.0	0.0330	0.0330	7.00	2.15
30.0	0.0198	0.0462	7.18	3.44
30.0	0.0066	0.0594	7.80	12.2[a]
35.0	0.0328	0.0008	5.18	2.43
35.0	0.0594	0.0066	5.91	2.69
35.0	0.046	0.0198	6.49	3.84
35.0	0.0462	0.0198	6.53	4.61
35.0	0.0198	0.0462	7.18	8.45
35.0	0.0066	0.0594	7.70	27.7[a,b]
35.0	0.0066	0.0595	7.76	24.6
35.0	0.0033	0.0627	8.04	38.0
35.0	0.0017	0.0643	8.32	60.7
35.0	0.0000	0.0660	9.00	200[b,c]
37.5	0.0198	0.0462	7.16	13.4
40.0	0.0328	0.0008	5.20	3.27
40.0	0.0462	0.0198	6.41	4.48
40.0	0.0330	0.0330	6.78	9.60
40.0	0.0198	0.0462	7.12	17.1
40.0	0.0100	0.0234	7.18	21.2[a,b]
40.0	0.0066	0.0594	7.67	56.8
45.0	0.0300	0.0034	5.70	4.98[a]
45.0	0.0234	0.0100	6.33	10.6[a]
45.0	0.0167	0.0167	6.68	22.1[a,c]
45.0	0.0100	0.0234	7.11	49.5[c]
45.0	0.0066	0.0594	7.65	120[a]
50.0	0.0300	0.0034	5.80	14.4
50.0	0.0234	0.0100	6.33	41.5
50.0	0.0167	0.0167	6.62	79.8
50.0	0.0100	0.0234	6.95	126
50.0	0.0034	0.0300	7.42	256
	$[CH_3COOH]$	$[CH_3COO^-]$		
35.0	0.037	0.003	3.05	2.56
35.0	0.033	0.007	3.51	2.51
35.0	0.025	0.015	4.02	2.35
35.0	0.020	0.020	4.29	2.38
35.0	0.008	0.030	4.90	2.30
35.0	0.004	0.036	5.39	2.28
50.0	0.020	0.020	4.39	9.00
	$[Na_2B_4O_7]$	[HCl]		
35.0	0.188	0.0495	8.42	67.0
35.0	0.119	0.0390	9.20	248
35.0	0.158	0.0198	9.30	347
	$[Na_2B_4O_7]$	$[Na_2CO_3]$		
35.0	0.025	0.0000	9.10	258
35.0	0.0161	0.0089	9.39	433
33.8	0.0062	0.0188	9.99	1150[d]
33.8	0.0033	0.0217	10.40	2780[d]
33.8	0.0021	0.0229	10.69	5220[d]
40.0	0.0250	0.0000	8.90	467[a]
45.0	0.0250	0.0000	8.90	997[a]

a) When the concentration of the phosphate buffer was varied, in some cases down to 0.1 of the stated values, no significant difference in the observed rate constant was observed.
b) When the concentration of the nitrosourea was varied, in some cases over a ten-fold range, no significant difference in the observed rate constants was observed.
c) When the ionic strength was varied in the range 0.10—0.40 with KCl, no significant difference in the observed rate constant was observed.
d) These values were obtained directly from the recording of absorbance as a continuous function of time in a thermostatted cell.

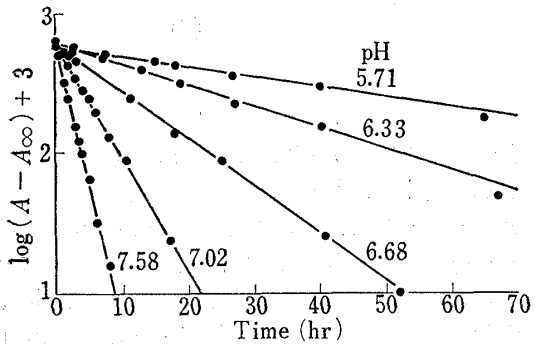


Fig. 2. Typical First Order Plots for the loss of Absorbance on Solvolysis of Nitrosoureas. The Loss of 230 mμ Absorbance of N,N'-Dimethyl-N-nitrosourea at 45.0°, μ=0.1 at Various pH Values

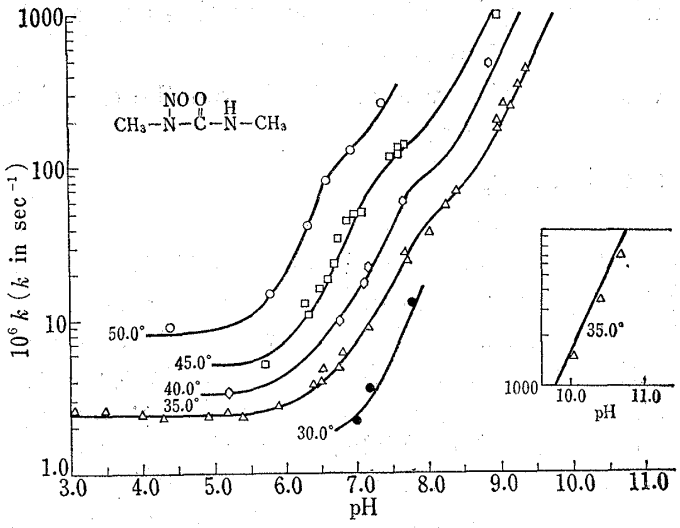


Fig. 3. Log k-pH Profiles for the Solvolysis of N,N'-Dimethyl-N-nitrosourea at Various Temperatures with k in sec^{-1}

profile (Fig. 3) *ca.* pH 8.20 at 35.0°. The log k-pH profiles in Fig. 3 for 35.0° were fitted[15] by

$$k = (k_0 + k_{OH} \cdot [OH^-]) f_{HA} + k'_{OH}[OH^-] f_{A^-} \quad (2)$$

where an equilibrium for the nitrosourea (HA) is postulated as:

$$HA \overset{K_a}{\rightleftharpoons} H^+ + A^- \quad (3)$$

The fraction of the nitrosourea, f_{HA}, that is presumed to be nonionized is calculated from[15]

$$f_{HA} = \frac{[H^+]}{[H^+]+K_a} \quad (4)$$

The fraction that is ionized is calculated from

$$f_{A^-} = \frac{K_a}{[H^+]+K_a} \quad (5)$$

Table II. Pertinent Constants, and the Microscopic Rate Constants[a] (k_{OH} and k'_{OH} in liter/mole sec, k_0 in sec^{-1}), Arrhenius Parameters[b] and pK_a values for the Solvolytic Degradation of N,N'-Dimethyl-N-nitrosourea Calculated from log k-pH Profiles

°C	pK_w	pK_a	$10^6 k_0$	k_{OH}	k'_{OH}
30.0	13.83	—	(1.60)[c]	10.8	—
35.0	13.66	8.20	2.40	22.9	6.5
40.0	13.54	(8.10)[d]	3.27	39.8	20.1[e]
45.0	13.40	(7.90)[d]	5.00	89.1	31.7[e]
50.0	13.26	—	9.00(8.0)[c]	269(182)[c]	(55.0)[c]
ΔH (kcal mole^{-1})			16.4	28.6	28.6
log P			6.0	20.9	21.4

a) Where $k_{apparent} = (k_0 + k_{OH}[OH^-]) f_{HA} + k'_{OH}[OH^-] f_{A^-}$ and $f_{HA} = [H^+]/([H^+]+K_a)$ and $f_{A^-} = K_a/([H^+]+K_a)$ and $[OH^-] = 10^{-pOH}$, $[H^+] = 10^{-pH}$ and $pOH = pK_w - pH$
b) where $\log k_1 = \Delta H_a/2.303\ RT + \log P$
c) estimated from Arrhenius plot of pertinent $\log k_1$ *vs.* 1/T
d) estimates only
e) based on one point only *i.e.*, k at pH *ca.* 9 and the expression $\log k'_{OH} = \log k_{apparent} - pH + pK_w$

15) E.R. Garrett, "Advances in Pharmaceutical Sciences," des. H.S. Bean, A.H. Beckett, and J.E. Carless, Vol. II, Academic Press, London, 1967, pp. 1—94.

where the hydroxyl ion concentration was calculated from

$$[OH^-] = 10^{-pOH} \quad (6)$$

where the pOH was obtained from the experimental pH and the literature pK_W (Table II)

$$pOH = pK_W - pH \quad (7)$$

and similarly,

$$[H^+] = 10^{-pH} \quad (8)$$

The excellent fits to the data of Table I are shown in Fig. 3 for 35.0° as based on the equation (2) using the estimates of the pertinent parameters given in Table II.

The data for the other temperatures were not as complete as for 35.0°. The general estimates of the specific rate constants k_0 and k_{OH} are also given in Table II. The values for k'_{OH} are very rough estimates based only on one point (Table I) obtained at higher alkalinities. These values have higher errors since the rates are fast but nevertheless indicate the decided presence of a perturbation in the linearity of the log k-pH profile that could be ascribed to a change in the charge on the nitrosourea in accordance with Eq. (3).

Spectrophotometric verification of a pK_a for this compound was obtained by studying the rapid decreases of absorbance of a constant concentration of NN'DMNU at 225 mμ at 35° at various pH values and obtaining zero time estimates of absorbance. In the pH range 8.8—10.0 the zero time absorbance values decrease with pH and are constant between pH 10 and 10.5, indicative of an apparent spectrally affected pK_a.

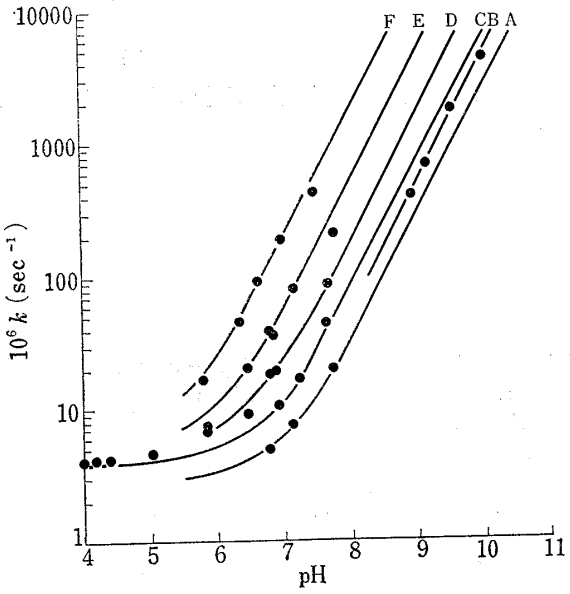


Fig. 4. Log k-pH Profiles for the Solvolysis of N-Methyl-N'-phenethyl-N-nitrosourea at Various Temperatures with k in sec^{-1}

A) 30.0°; B) 33.6°; C) 35.0°; D) 40.0°; E) 45.0°; F) 50.0°

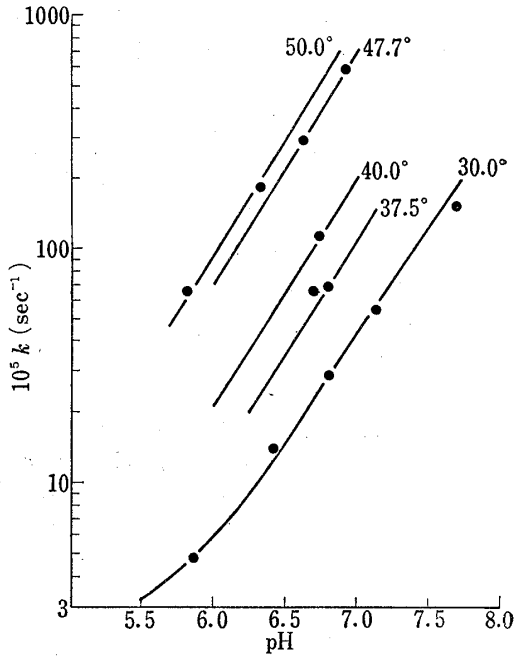


Fig. 5. Log k-pH Profiles for the Solvolysis of N-Methyl-N'-p-fluorophenyl-N-nitrosourea at Various Temperatures with k in sec^{-1}

Kinetics of N-Methyl-N'-phenylethyl-N-nitrosourea, N-Methyl-N'-*p*-fluorophenyl-N-nitrosourea and Trimethylene bis N-Methyl-N-nitrosourea Degradation

The log k-pH profiles for the solvolysis of N-methyl-N'-phenethyl-N-nitrosourea, N-methyl-N'-p-fluorophenyl-N-nitrosourea and trimethylene bis-N-methyl-N-nitrosourea are given in Fig. 4, 5 and 6 respectively as based on the data of Table III, IV and V. The data can be readily fitted with the equation

$$k = k_0 + k_{OH}[OH^-] \quad (9)$$

where the hydroxide ion concentration is obtained from Eq. (6) and (7). The method used was to obtain an estimate of k_0 and from the plot of log $(k-k_0)$ *vs* pH of slope unity, the resultant intercept had the value of log k_{OH}—pK_W. Thus log k_{OH} can be calculated from the knowledge of pK_W[16] at the pertinent temperature (Table II). This is based on the expression

$$\log (k-k_0) = \log k_{OH}[OH^-] = \log k_{OH}k_W/[H^+]$$
$$= \log k_{OH} - pK_W + pH \qquad (10)$$

The pertinent microscopic rate constants are given in Table VI for the various temperatures and the lines drawn through the data of Fig. 4—6 are based on those constants.

In contrast to the N′-methyl compound the log k—pH profiles of these compounds did not indicate any apparent kinetic pK_a values, at least up to the highest pH values studied.

Kinetics of Solvolysis of 1,6-Dimethyl-1,6-dinitrosobiurea

The apparent first order rate constants for the solvolysis of 1,6-dimethyl-1,6-dinitrosobiurea at various pH values are listed in Table VII and the log k—pH profile at 35.0° is plotted in Fig. 7. Hydrogen-ion catalyzed solvolysis below a pH of 3 and hydroxyl ion catalyzed solvolysis above a pH of 5 on the uncharged species is apparent from the plot.

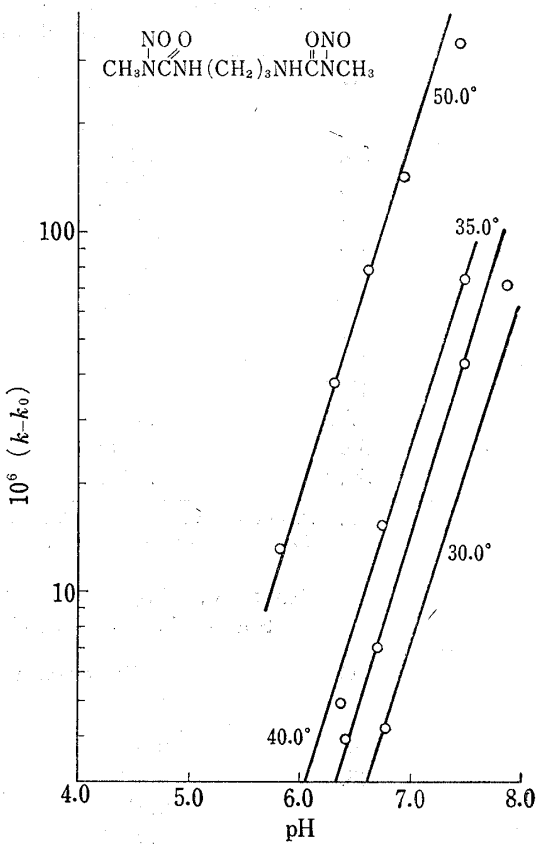


Fig. 6. Log $(k-k_0)$-pH Profiles for the Solvolysis of Trimethylene bis-N-Methyl-N-nitrosourea at Various Temperatures with k and k_0 in sec^{-1}

The k_0 values are for apparent pH-independent solvolysis and are for 10^6k_0: 1.3 at 30°, 2.5 at 35°, 5.0 at 40° and 30 at 50°.

Table III. Apparent First Order Rate Constants (k in sec^{-1}) for the Solvolysis of 10^{-4}M N-Methyl-N′-phenethyl-N-nitrosourea

°C	pH	10^6k	°C	pH	10^6k	°C	pH	10^6k	°C	pH	10^6k
30.0	6.79[a]	4.80	35.0	3.80[b]	4.10	40.0	6.45[a]	9.06	45.0	6.82[a]	37.1
	7.13[a]	7.55		4.20[b]	4.25		6.80[a]	18.4		7.14[a]	82.5
	7.71[a]	19.8		4.40[b]	4.26		6.86[a]	19.3		7.72[a]	217
33.8	8.90[c]	440		5.03[b]	4.64		7.67[a]	88.7	50.0	5.82[a]	16.8
33.6	9.12[c]	709		6.52[a]	3.80	45.0	5.86[a]	6.72		6.33[a]	47.2
	9.46[c]	1890		6.90[a]	10.4		5.88[a]	7.23		6.62[a]	93.3
	9.96[c]	4560		7.21[a]	16.8		6.43[a]	20.7		6.95[a]	198
35.0	3.31[b]	5.25		7.61[a]	46.0		6.79[a]	39.0		7.42[a]	445

a) $H_2PO_4^-$—$HPO_4^=$ buffer system
b) $HC_2H_3O_2$–$C_2H_3O_2^-$ buffer system
c) $Na_2B_4O_7$–Na_2CO_3 buffer system. These studies were conducted by recording absorbance as a continuous function of time in a thermostatted cell.

The deviation from linearity between pH values of 7.5 and 10 can be readily rationalized by the assumption that the compound is a dissociable acid with a pK_a' of 7.4. The plotted curve through the values of Fig. 7 is fitted to the kinetic dependency of

16) H.S. Harned, and B.B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd Ed., Reinhold Publishing Co., New York, N.Y., 1958.

Table IV. Apparent First Order Rate Constants (k in sec^{-1}) for the Solvolysis of 10^{-4}M N-Methyl-N′-*p*-fluorophenyl-N-nitrosourea

°C	Buffer composition		pH	10^6k
	$[H_2PO_4^-]$	$[HPO_4^=]$		
30.0	0.0300	0.0034	5.86	48.0
30.0	0.0234	0.0100	6.42	139
30.0	0.0167	0.0167	6.80	283
30.0	0.0100	0.0234	7.14	553
30.0	0.0034	0.0300	7.69	1550
35.0	0.0167	0.0167	6.68	663
37.5			6.79	698
40.0			6.73	1150
47.7			6.62	2920[a]
47.7	0.0100	0.0234	6.91	5940[a]
50.0	0.0300	0.0034	5.81	659
50.0	0.0284	0.0100	6.32	1860

a) These values were obtained directly from the recording of absorbance as a continuous function of time in a thermostatted cell.

Table V. Apparent First OrderRate Constants (k in sec^{-1}) for the Solvolysis of 10^{-4}M Trimethylene bis-N-Methyl-N-nitrosourea

°C	Buffer composition		pH	10^6k
	$[H_2PO_4^-]$	$[HPO_4^=]$		
30.0	0.0167	0.0167	6.79	5.36
35.0	0.0234	0.0100	6.40	6.40
	0.0167	0.0167	6.72	9.53
	0.0034	0.0300	7.52	46.1
	0.0017	0.0317	7.87	76.7
40.0	0.0234	0.0100	6.40	10.1
	0.0167	0.0167	6.72	19.7
	0.0084	0.0084	6.74	22.4
	0.0035	0.0035	6.78	28.1
	0.0034	0.0300	7.52	85.4
50.0	0.0300	0.0034	5.81	43.0
	0.0234	0.0100	6.32	68.0
	0.0167	0.0167	6.62	109
	0.0100	0.0234	6.91	173
	0.0004	0.0300	7.42	378

Table VI. Microscopic Rate Constants[a] (k_{OH} in liter/mole-sec; k_0 in sec^{-1}) for the Solvolytic Degradation of Variously substituted Nitrosoureas Calculated from Log k-pH Profiles

°C	N-Methyl-N′-phenethyl-N-		N-Methyl-N′-*p*-fluorophenyl-N-		Trimethylene bis-N-Methyl-N-	
	10^6k_0	k_{OH}	10^6k_0	k_{OH}	10^6k_0	k_{OH}
30.0	3.0	22.4	20	2690	—	—
33.6	3.7	31.0	—	—	—	—
35.0	4.1	37.2	—	—	2.5	61.7
37.5	—	—	—	4570	—	—
40.0	5.0	67.7	—	7250	5.0	91.2
45.0	5.0	139	—	15900	—	—
50.0	6.0	355(235)[b]	—	17400	30	324
ΔH_a[c]	—	23.0	—	18.8	—	22.6
log P[c]	—	17.9	—	17.0	—	17.8

a) Where k (in sec^{-1}) $=k_o+k_{OH}[OH^-]$, $[OH^-]=10^{-pOH}$, and $pOH=pK_w-pH$. Thus, $\log(k-k_o)=\log k_{OH}[OH^-]=\log k_{OH}-pK_w+pH$ where the pK_w values for the several temperatures are listed in Table II.

b) Estimated from the Arrhenius plot of $\log k_{OH}$ *vs.* $1/T$ where $\log k_{OH}=\Delta H_a/2.303RT+\log P$ where ΔH_a is in kcal/mole.

TABLE VII. Conditions and Apparent First Order Rate Constants (k in sec^{-1}) for the Solvolysis of 10^{-4}M 1,6-Dimethyl-1,6-dinitrosobiurea

°C	Buffer composition		pH	10^6k
	$[CH_3COOH]$	$[CH_3COO^-]$		
35.0	0.095	0.003	3.11	2.50
	0.0495	0.0495	4.50	3.70
	0.0198	0.0792	5.10	5.00
	0.003	0.095	6.00	25.3
	$[H_2PO_4^-]$	$[HPO_4^=]$		
35.0	0.0594	0.0066	5.80	19.5
	0.0462	0.0198	6.37	59.2
	0.0330	0.0330	6.70	125
	0.0198	0.0462	7.01	212
	0.0066	0.0594	7.60	492
	0.0017	0.0643	8.19	772
	—	0.0660	8.56	795[a]
	—	0.1000	8.92	915
40.0	0.0330	0.0330	6.58	203
37.5	0.0330	0.0330	6.60	152
30.0	0.0330	0.0330	6.60	56.0
	$[H_2PO_4^-]$	$[B_4O_7^=]$		
35.0	0.017	0.041	9.10	837[a]
35.0	0.005	0.047	9.20	1130[a]
	[NaOH]			
35.0	0.00083		10.60[b]	2320[a]
	0.0013		10.79[b]	3740[a]
	0.0021		11.05[b]	4600[a]
	0.0032		11.23[b]	7220[a]
	0.0042		11.35[b]	6750[a]
	0.0064		11.49[b]	9560[a]
24.5	0.0013		11.12[b]	2840[a]
	0.0032		11.52[b]	5950[a]
	0.0064		11.82[b]	10600[a]

a) SRL recorder used.
b) Calculated from $pH=pK_w-pOH=pK_w+\log f_{NaOH}[NaOH]$ where the pK_w and the f_{NaOH} as the mean activity coefficients of the sodium hydroxide solution are obtained from the literature (16)

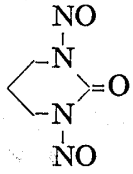
$$k = k_H[H^+] + k_0 + k_{OH}[OH^-]f_{HA} + k'_{OH}[OH^-]f_{A^-} \quad (11)$$

where the fractions, f_{HA} and f_{A^-} of nonionized and ionized species respectively, are calculated from the relations of Eq. 4 and 5. The values of the $\log k_{OH}=3.0$ and $\log k'_{OH}=0.21$ were estimated by application of Eq. (10) to the linear segments of positive unit slopes in the alkaline branch of the log k—pH profile. The $\log k_H=-3.13$ was estimated from the intercept of the linear segment of negative unit slope in the acid branch as based on the expression:

$$\log k = \log k_H[H^+] = \log k_H - pH \quad (12)$$

Thus the values of the constants at 35.0° are $k_0=2.5\times10^{-6}$ in sec^{-1} and $k_H=7.5\times10^{-4}$, $k_{OH}=1000$ and $k'_{OH}=1.62$ in liter/mole·sec.

Kinetics of Solvolysis of Tetrahydro-1,3-dinitroso-2(1H)-pyrimidinone

The apparent first order rate constants obtained by monitoring the UV absorbance at 260 mμ at various phosphate buffer concentrations and pH values is given for tetrahydro-1,3-dinitroso-2(1H)-pyrimidinone  at 35.0° in Table VIII. A plot of log k against pH is not unity and showed significant variation in the k values with varying phosphate buffer

concentrations at the same pH value. The fact that the apparent first order rate constants varied widely with phosphate buffer concentration at a higher pH value where the major contribution is from the monohydrogen phosphate dianion and negligibly at a lower pH value where the major contribution is from the dihydrogen phosphate monoanion implicates the dianion in catalysis in accordance with the expression

$$k = k_0 + k_{HPO_4}[HPO_4^{2-}] + k_{OH}[OH^-] \quad (13)$$

Specific monohydrogen phosphate catalysis has been observed also in the solvolysis of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and 1,3-bis-(2-chloroethyl)-1-nitrosourea.[13]

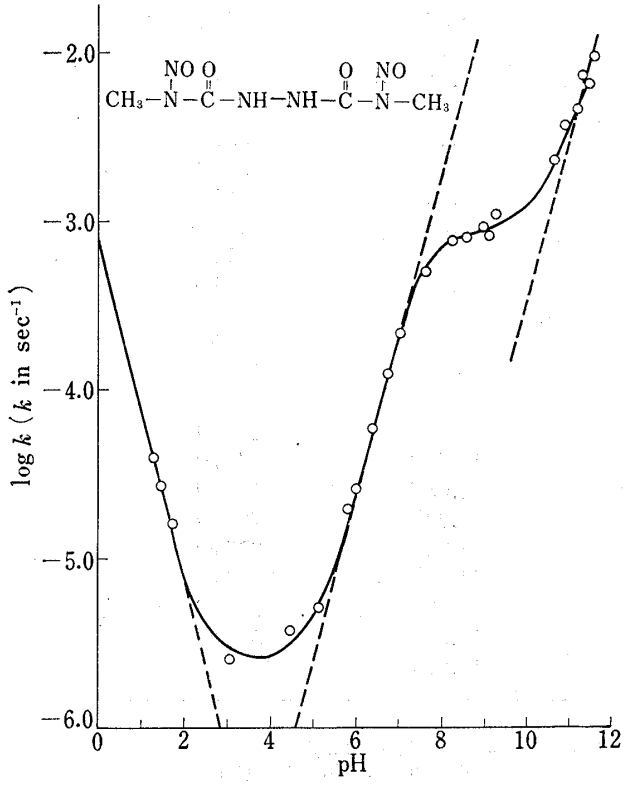


Fig. 7. Log k-pH Profile for the Solvolysis of 1,6-Dimethyl-1,6-dinitrosobiurea at 35.0° with k in sec^{-1}

Table VIII. Rate Constants (k in sec^{-1}) for the Apparent First Order Solvolysis of Tetrahydro 1,3-dinitroso-2 (1H)-pyrimidinone at 35.0° and 0.100 Ionic Strength

pH	Buffer composition $[H_2PO_4^-]$	$[HPO_4^=]$	10^4k
4.99	0.0325	0.00083	1.17
4.99	0.0163	0.00042	1.17
5.00	0.0082	0.00021	1.17
5.41	0.0300	0.0034	1.10
5.40	0.0150	0.0017	1.00
5.38	0.0075	0.0009	1.01
6.41	0.0234	0.0100	3.06
6.41	0.0117	0.0050	2.36
6.40	0.0059	0.0025	2.00
6.60	0.0084	0.0084	3.64
6.59	0.0084	0.0084	3.64
6.59	0.0042	0.0042	3.32
7.23	0.0100	0.0234	12.5
7.18	0.0056	0.0117	10.8
7.51	0.0034	0.0300	18.7
7.49	0.0017	0.0150	17.3

When the apparent first order rate constant, k, was plotted against $[HPO_4^=]$ at the several pH values, the slope (k_{HPO_4}) and intercepts ($k_0+k_{OH}[OH^-]$) were: pH, pOH, k_{HPO_4}, $k_0+k_{OH}[OH^-]$; 7.50, 6.16, 1.00×10^{-2} (liter/mole·sec), 15.9×10^{-4}(sec^{-1}); 7.20, 6.46, 1.44×10^{-2}, 9.18×10^{-4}; 6.59, 7.07, 1.26×10^{-2}, 3.02×10^{-4}; 6.41, 7.25, 1.41×10^{-2}, 1.66×10^{-4}; 5.40, 8.26, 0.0, 1.00×10^{-4}; 5.00, 8.66, 0.0, 1.17×10^{-4} where $pK_w=13.66=pH+pOH$ at 35.0°. Thus an average value of k_{HPO_4} at 35.0° is 1.3×10^{-2} liter/mole·sec. A plot of the resultant intercepts ($k_0+k_{OH}[OH^-]$) against the hydroxyl ion concentration calculated from Eq. (6) gave a straight line of slope, $k_{OH}=213$ liter/mole·sec and an intercept of 1.15×10^{-4} sec^{-1} for k_o. This latter value is coincident with the observed apparent first order rate constants at pH 5.00 where no phosphate buffer effect was observed and hydroxyl ion catalyzed solvolysis is apparently negligible.

Arrhenius Parameters

Wherever possible the Arrhenius parameters were determined from the microscopic rate constants in accordance with the expression

$$\log k_i = -\Delta H_a/2.303RT + \log P \quad (14)$$

where T is in degrees Kelvin and R is 1.986 cal deg^{-1} mole^{-1}. Values for the pertinent N-nitrosourea are given in Table II and VI. In other cases the parameters were obtained from Arrhenius plots of apparent first order rate constants at pH 6.80. These are given for various nitrosoureas in Fig. 8 for purposes of comparison. The structures of these compounds are also given in the Figures.

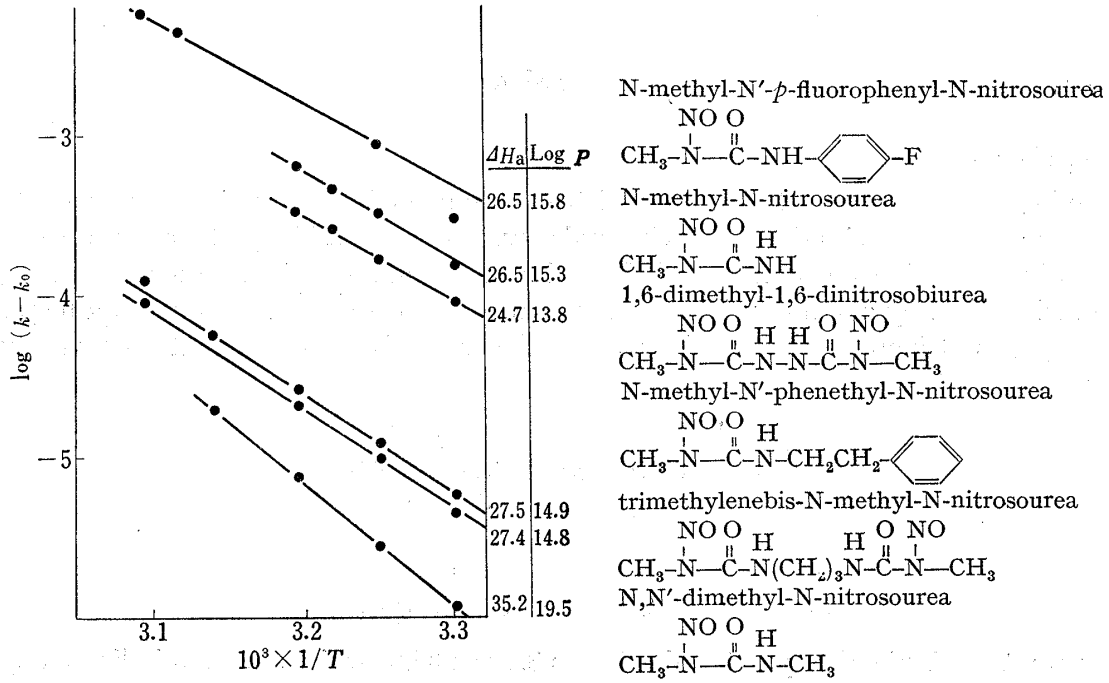


Fig. 8. Arrhenius Plots and Parameters of Apparent First Order Rate Constants for the Solvolysis of Various N-Nitrosoureas at pH 6.80

Investigation of the Degradation Products of N-Nitrosoureas by Gas Chromatography

The solutions of mixtures of *n*-butyl alcohol, isobutyl alcohol, secondary butyl alcohol and tertiary butyl alcohol were analyzed on the gas chromatograph with the flame ionization detector under the experimental conditions specified. No signals were observed with the electron capture detector. These conditions gave clean separations of each alcohol with clearly defined peaks and the retention time decreased in the order given. The retention times were obtained from studies on each alcohol separately. Good linear plots of peak heights of injected ether extract against concentration in the aqueous solution were obtained for each alcohol in the mixture and were used as calibration curves.

The degradations of N-nitroso-N-butylurea conducted at various pH values of 7.27, 9.05, 10.85 and 12.30 overnight showed no significant differences among the peaks observed by flame ionization or in their peak heights. The peaks corresponded to *n*-butyl alcohol with an average yield of 45% and secondary butyl alcohol with an average yield of 29%.

The products of alkaline degradation were benzoylated and the injected solutions prepared showed peaks with the same retention time as the benzoylated normal and secondary butyl alcohols. These were at different retention times than the non derivatized alcohols that were the product of the N-butyl-N-nitrosourea degradation and thus clearly showed that both alcohols were obtained on alkaline degradation.

The degraded 1.22×10^{-2}M N-isobutyl-N-nitrosourea at pH 12.30 (0.1M NaOH) was monitored gas chromatographically and demonstrated peaks consistent with *t*-butyl alcohol (76%), *sec*-butyl alcohol (22%) and isobutylalcohol (18%) where the parenthetical values are the percentage yields of each alcohol based on the calibration curves prepared for alcohol mixtures for peak height ratios with the internal standard dodecane.

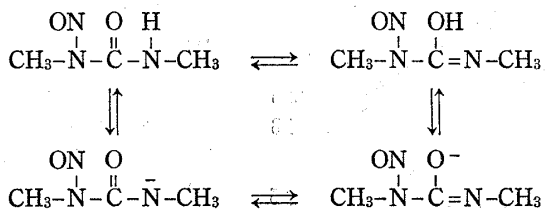
Degraded 10^{-2}M N-allyl-N-nitrosourea at pH 12.30 (0.1M NaOH) showed only one peak, consistent with an 87% yield of allyl alcohol based on comparison with a calibration curve of peak height ratio against concentration prepared for allyl alcohol with the internal standard.

Degraded 1.23×10^{-2}M N-benzyl-N-nitrosourea at pH 12.30 (0.1M NaOH) showed only one peak consistent with a 120% yield of benzyl alcohol based on comparison with a calibration curve of peak height ratio against concentration prepared for benzyl alcohol with the internal standard.

Discussion

Log k—pH Profile

The log k—pH profile of N,N′-dimethyl-N-nitrosourea (Fig. 3 and Table I and II) indicated that the neutral species degraded with hydroxyl ion attack and that there was a lessened attack of hydroxyl ion on some modified species in alkaline solution. Such a phenomenon can be rationalized by assuming that the nitrosourea dissociates to a proton and a negatively charged species at increasing alkalinities where the latter is more resistant to hydroxyl ion attack:



The apparent pK_a', kinetically determined, was 8.20 at 35.0° which was also indicated by spectral measurements of rate studies at various pH values extrapolated to zero time. However, it is admitted that these are very fast reactions and these extrapolated data may not be too relative to estimate a spectrophotometric pK_a'.

The N-methyl-N′-phenethyl, N-methyl-N′-*p*-fluorophenyl-N-nitrosoureas (Fig. 4 and 5, Table III and IV) trimethylene bis-N-methyl-N-nitrosourea (Fig. 6, Table V) and tetrahydro 1,3-dinitroso-2 (1H)-pyrimidinone (Table VIII) show no such apparent kinetic pK_a'. However, all of these compounds, except the first were studied only below pH 8.0.

The log k—pH profile of 1,6-dimethyl-1,6-dinitrosobiurea also exhibited an apparent kinetic pK_a' (Fig. 7 and Table VII), of 7.4 at 35.0°. Potentiometric titration geve an apparent pK_a' consistent with this value for this compound. No apparent pK_a's were obtained on titrating the other nitrosoureas.

Comparison of Reactivities of 1-Substituted and 1,3-Disubstituted Nitrosoureas

The substitution of an alkyl on the N′-nitrogen decreases the rates of hydrolyses of the neutral N-methyl-N-nitrosoureas. The relative reactivities of several of these compounds are readily seen from the Arrhenius plot of Fig. 8. For example, whereas the bimolecular rate constants for hydroxyl ion attack at 35.0° for $R\text{-}\overset{\overset{NO}{|}}{N}\text{—}\overset{\overset{O}{\|}}{C}\text{-}NH_2$[12] are 2380 for R=methyl and 4300 L/M·sec for R=phenethyl; for $CH_3\text{-}\overset{\overset{NO}{|}}{N}\text{—}\overset{\overset{O}{\|}}{C}\text{-}NHR'$ (Table VI) it is 22.9 for R′=methyl, 37.2 for R′=phenethyl. This is approximately a ten-fold decrease in relative reactivity for the introduction of an N′-substituent. The sequences of activities, however, in both series[12] are reasonably consistent with the fact that electron withdrawing properties of the substituents abet hydroxyl ion attack by inducing a more positive center. The higher reactivity for R′=*p*-fluorophenyl ($k_{OH}\simeq4000$ at 35.0°) conforms to this assertion. The fact that an R′ substituent inhibits the hydrolysis can be rationalized in that substitution of an alkyl for a hydrogen

on the N′-nitrogen should significantly lower the positive polarity of the adjacent carbonyl carbon which is thus favored to be the primary focus of attack of the hydroxide ion.[13]

Products and Mechanisms of Alkaline Solvolysis of N-Alkyl-N-nitrosoureas

The retention times of the gas chromatographically analyzed solutions of the alkaline degraded N-alkyl-N-nitrosoureas clearly showed consistency with standard alcohols that could be anticipated as possible products. In the specific case of alkaline degradation of N-nitroso-N-butylurea the benzoyl esters of the degradation products were prepared. They had different retention times than the original alcohols but the same as the retention times of the standard benzoyl esters. This provided definitive proof of their identity. Also, there was no significant difference among the kinds and yields of alcohols from the alkaline degradation of this compound above pH 7.3.

The facts that the alkaline degradation products of N-*n*-butyl-N-nitrosourea were *n*-butyl alcohol (45%) and secondary butyl alcohol, that the products of N-isobutyl-N-nitrosourea were *t*-butyl alcohol (76%), *sec*-butyl alcohol (22%) and isobutyl alcohol (18%), imply that electron and methyl shifts occur in the reaction processes. The implication is that in the N-*n*-butyl-N-nitrososurea case, the intermediate product is the *n*-butyl carbonium ion which equilibrates its charge with the secondary carbon to produce both primary and secondary alcohols via the shift of a hydride ion.

$$CH_3\text{–}CH_2\text{–}CH_2\text{–}CH_2^+ \rightleftharpoons CH_3CH_2\text{–}\underset{+}{CH}\text{–}CH_3$$

Similarly, the N-isobutyl-N-nitrosourea produces a carbonium ion that shows electron and methyl shifts.

$$CH_3\text{–}\underset{+}{CH_2}\text{–}CH_2\text{–}CH_3 \rightleftharpoons CH_3\text{–}\overset{CH_3}{\overset{|}{\underset{+}{CH}}}\text{–}CH_2 \rightleftharpoons CH_3\text{–}\overset{CH_3}{\overset{|}{\underset{+}{C}}}\text{–}CH_3$$

which can account for the alcohols with the observed rentention times. These are phenomena characterized by carbonium ion formation and have been well demonstrated inFriedel-Kraft type reactions and the action of alkyl halides with aluminum chloride.[17] Thus, the probable diazoalkane product in N-nitrosourea degradation most probably goes through a carbonium ion intermediate, *e.g.*

$$CH_3\text{–}CH_2\text{–}CH_2\text{–}CH_2\text{–}N{=}N^+ \longrightarrow CH_3\text{–}CH_2\text{–}CH_2\text{–}CH_2^+ + N_2$$

As would be expected, since there is no available neighboring methylene group in N-alkyl-N-nitrosourea and N-benzyl-N-nitrosourea, the alkaline solvolyses of these compounds yielded only the expected alcohol.

17) L.F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Co., New York, N.Y., 1961, pp. 653—654.