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Studies on Chemical Carcinogens. XXIII.[1] A Simple Method for Characterization of the Alkylating Ability of Compounds by using 4-(*p*-Nitrobenzyl)pyridine

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The present work provides a simple method for the characterization of alkylating agents by measuring the reaction selectivity toward 4-(*p*-nitrobenzyl)pyridine (NBP) in acetone containing phosphate buffer, evaluated in terms of the selectivity constant, S_{NBP}, which is defined as log $\{[H_2O]/[NBP] \times N\%/(100-N\%)\}$, where $N\%$ is the percentage molar fraction of NBP alkylation. S_{NBP} values were determined for about 40 kinds of halides, methanesulfonates, tosylates, sulfates, phosphates, nitrosoureas, and nitrosoguanidines. S_{NBP} can replace the substrate constant, s, of the Swain–Scott equation, and S_{NBP}'s of some of the agents examined here were linearly correlated with the s values.

Keywords——alkylating agents; 4-(*p*-nitrobenzyl)pyridine; NBP; regioselectivity; substrate constant; selectivity constant

In recent years, attention has been focused on the alkylating ability of synthetic and environmental carcinogens and mutagens, because their biological activities are thought to be initiated by alkylations of cellular desoxyribonucleic acid (DNA).[2,3] Thus, it is probable that carcinogenic and mutagenic potencies are significantly related to the chemical properties of the alkylating agents concerned, *i.e.*, the alkylation rate and the regioselectivity toward nucleophilic centers present in biological molecules.[2] Since Koenigs *et al.* reported[4] in 1925 that 4-(*p*-nitrobenzyl)pyridine (NBP) reacted with methyl iodide to give the salt, which was converted upon addition of potassium hydroxide to a colored material, as formulated in Chart 1, this reaction has often been applied to detect the alkylating ability of compounds. The experimental procedure has been developed and improved by Epstein *et al.*,[5] Wheeler *et al.*,[6] and Druckrey *et al.*[7] All the procedures so far reported consist of the treatment of an alkylating agent with NBP in a mixture of a buffer and an organic solvent for a certain period of time (30 min in most cases) at room temperature or at the boiling temperature of the solvents used, followed by making the reaction mixture alkaline with sodium hydroxide, potassium carbonate, triethylamine, or piperidine. The optical density of the resultant intensely colored reaction mixture is immediately measured with a spectrophotometer for quantitative analysis of the alkylating agent which reacted with the excess NBP. It seems, however, that there are many deficiencies in these procedures for the characterization of alkylating agents in terms of the chemoselectivity and the reaction rate. The substrate constant in nucleophilic substitution reactions, defined by Swain and Scott,[8] may give a measure of the chemoselectivity of alkylating agents. However, it is tedious to perform many kinetic measurements to evaluate the substrate constant of various alkylating agent possibly involved in carcinogenesis and mutagenesis. The present paper describes a simple method for the characterization of alkylating agents in terms of the selectivity constant toward NBP, denoted as S_{NBP}. The selectivity

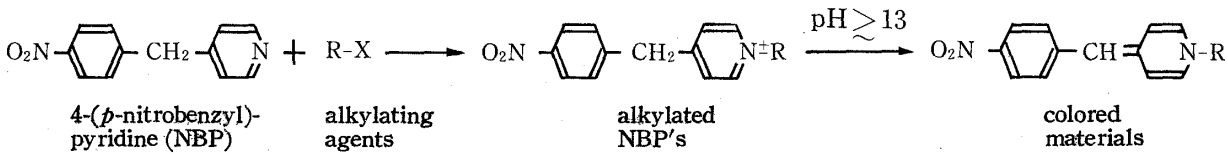

Chart 1. Formation of Colored Material by Alkylation of 4-(*p*-Nitrobenzyl)pyridine (NBP)

constants of a variety of carcinogens and mutagens are given. The relation between S_{NBP} and the Swain–Scott's substrate constant is discussed.

Experimental

Materials——Most of the alkylating agents used were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo). Some of the alkylnitrosoureas,[9] alkyl methanesulfonates,[10] alkyl tosylates,[11] and *p*-substituted benzyl halides[12,13] were synthesized by the reported methods. Other alkylating agents and alkylated NBP's were prepared as follows.

p-Acetamidobenzyl Chloride: *p*-Acetamidobenzyl alcohol (3.0 g) was dissolved in 300 ml of $CHCl_3$. After addition of 10 g of anhydrous $CaCl_2$, the reaction mixture was saturated with dry HCl gas. After 10 min at room temperature, the solvent was evaporated off to afford a colorless solid, which was recrystallized from benzene to give white crystals. mp 149—151°C. The yield was 70%. *Anal.* Calcd for $C_9H_{10}ClNO$: C, 58.87; H, 5.49; N, 7.63. Found: C, 59.34; H, 5.51; N, 7.24.

p-Acetamidobenzyl Bromide: In the same way as for the corresponding chloride, the bromide was prepared with HBr gas instead of HCl. mp 163—165°C. *Anal.* Calcd for $C_9H_{10}BrNO$: C, 47.39; H, 4.42; N, 6.14. Found: C, 47.57; H, 4.47; N, 6.15.

Alkylated NBP's: An alkylating agent (0.5—2.0 g) was refluxed with 0.5—2.0 g of NBP in about 30 ml of acetone for about 24 h. The crystalline material thus separated was washed thoroughly with acetone and dried over P_2O_5. Most of the alkylated NBP's did not require further purification for elementary analyses and nuclear magnetic resonance (NMR) measurements. None of them showed a sharp melting point, and melting was accompanied by gradual decomposition above 150°C. Analytical data are given in Table I.

Table I. Analytical Data for Alkylated 4-(*p*-Nitrobenzyl)pyridines (NBP)

Alkylated NBP	Appearance	Analytical data					
		Calcd			Found		
		C	H	N	C	H	N
Methyl-NBP iodide	Pale yellow needles	43.84	3.68	7.87	43.84	3.69	7.50
Pentyl-NBP iodide	Reddish solid	49.52	5.13	6.80	49.59	5.18	6.88
Allyl-NBP bromide	Pale yellow needles	53.70	4.51	8.36	53.64	4.57	8.44
Benzyl-NBP bromide	White scales	59.24	4.45	7.27	59.00	4.20	7.26
p-Methylbenzyl-NBP bromide	Pale yellow powder	60.11	4.80	7.02	60.03	5.02	6.70
p-Methoxybenzyl-NBP bromide	Pale yellow needles	57.80	4.61	6.75	57.40	4.43	6.24
p-Nitrobenzyl-NBP bromide	Pale yellow needles	53.00	3.75	9.77	52.59	3.58	9.54
p-Chlorobenzyl-NBP bromide	Pale yellow scales	54.37	3.84	6.67	54.32	4.04	6.50
α-Methyl-*p*-nitrobenzyl-NBP bromide	Pale yellow needles	54.02	4.08	9.46	54.07	4.21	9.21

Standard Analytical Procedure for Alkylating Ability by using 4-(*p*-Nitrobenzyl)pyridine (NBP)——The reaction mixture consists of 20 ml of 1/15 M phosphate buffer (pH 6.0), 20 ml of 0.25 M NBP (5×10^{-3} mol) in acetone, and 10 ml of acetone solution of an alkylating agent exactly weighed in the range of 10^{-4} to 5×10^{-6} mol (usually 1.36×10^{-5} mol). About 1.5 ml of the reaction mixture is placed in each of 10 to 15 small tubes and the tubes are sealed under an argon or nitrogen atmosphere. These tubes are warmed at 37°C and worked up one by one at appropriate intervals. Thus, 1.0 ml of the content of a tube is added to 4 ml of water and 5 ml of benzene and thoroughly mixed with a vibromixer after addition of 0.5 ml of 1 N NaOH. The wine-colored benzene layer is taken up with a pipette and dried over KOH pellets. The optical density (OD) of the solution is measured at the λ_{max} of the peak around 520—535 nm. The wine-colored compound thus extracted is quite stable in the absence of moisture, CO_2, and light. The OD finally reaches a plateau. From the maximum OD thus obtained, the ratio of alkylation of NBP is calculated on the basis of the molar extinction coefficient previously determined with an authentic specimen of each alkylated NBP.

Results

Quantitative Analysis of NBP Alkylation and the Selectivity Constant toward NBP, S_{NBP}, as a Measure of the Chemoselectivity of Alkylating Agents

From the results obtained in the present study, it is concluded that the standard analytical procedure described in the experimental section can be applied to characterize a given alkylating agent with regard to both the chemoselectivity and the reaction rate. It was experimentally confirmed that the amount of the alkylating agent could be varied from 1×10^{-4} to 5×10^{-6} mol depending on the efficiency of their alkylation of NBP and that the concentration of NBP recommended in the standard procedure, 0.1 M, could be reduced to 1/2 or less when the selectivity toward NBP was too large. Measurements of the time-course changes in NBP alkylation give the half-life ($t_{1/2}$) of the alkylating agent, which is a relative measure of the rate of the alkylation, as illustrated in Fig. 1. The molar fraction of alkylated NBP at the end of the alkylation reaction is calculated from the optical density of the colored material with reference to the initial amount of the alkylating agent used. The molar fraction in percent thus obtained is abbreviated as $N\%$ for the alkylation of nitrogen in the NBP molecule and the residual fraction, $(100-N\%)$, is that of all the other accompanying reactions. It is thought that the latter reactions are mainly alkylations of the oxygen functions in the reaction medium (OH^-, H_2O, and phosphate ions) and, in some cases, eliminations reactions to the ethenic products in a minor part. The selectivity constant toward NBP, termed S_{NBP}, is defined in this paper as follows, where the minor side reactions are tentatively ignored for convenience of formulation.

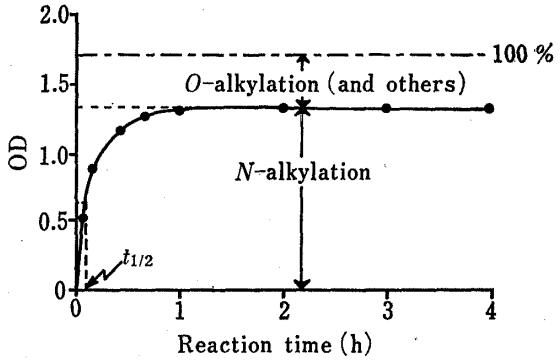

Fig. 1. Time Course Changes in the OD of the Benzene Extract from the Reaction of Benzyl Tosylate according to the Standard Analytical Method described in Experimental

The OD value expected for quantitative alkylation with all the benzyl tosylate used is 1.75 as indicated by the broken line (100%). The half-life can be roughly read from the figure as indicated.

$$S_{NBP} = \log\left\{\frac{[H_2O]}{[NBP]}\times\frac{N\%}{(100-N\%)}\right\}$$

In the standard analytical procedure described in this paper, where $[H_2O]$ is 22.2 M and [NBP] is 0.1 M,

$$S_{NBP} = \log\left[222\times\frac{N\%}{(100-N\%)}\right]$$

Using this procedure, the values of $N\%/(100-N\%)$ at [NBP]=0.1 M and the selectivity constants, in addition to the half-lives, of a variety of alkylating agents were experimentally obtained. They are listed in Table II.

The S_{NBP} value is not appreciably dependent on the concentration of NBP used for the analysis, although $N\%$ does vary with changes in the NBP concentration. Some data are shown in Table III. When the selectivity toward NBP is as large as almost 100% under the standard conditions, the amount of NBP can be reduced to 1/2 or less in order to determine S_{NBP} more accurately.

With regard to the dependence of $N\%$ on the pH of the phosphate buffer used for the analysis, no difference in $N\%$ was found in most cases between pH 5.0 and 7.0. This result

Table II. Half-lives of Alkylating Agents in Buffered Acetone containing 0.1 M 4-(*p*-Nitrobenzyl)pyridine (NBP) at 37°C and Selectivity Constants toward NBP, S_{NBP}

Alkyl group	Leaving group	Half-life[a] (h)	N%[b]	S_{NBP}[c]
Halides				
CH_3–	–I	2.5	99	4.34
CH_3CH_2–	–I	35	99	4.34
$CH_3(CH_2)_3$–	–Cl	90	13	1.52
	–Br	104	99	4.34
	–I	27	99	4.34
$CH_3(CH_2)_4$–	–I	26	99	4.34
$(CH_3)_2CH$–	–I	82	22	1.80
$(CH_3)_2NCH_2CH_2$–	–Cl	1.2	92	3.41
	–Br	1.2	96	3.73
C_6H_5–CH_2–	–Br	0.5	99	4.34
p-CH_3O–C_6H_4–CH_2–	–Cl	0.08	4.0	0.97
	–Br	0.08	9.0	1.34
p-CH_3CONH–C_6H_4–CH_2–	–Cl	0.42	8.0	1.29
	–Br	0.08	24	1.85
p-NO_2–C_6H_4–CH_2–	–Cl	41.0	88	3.21
	–Br	1.0	99	4.34
C_6H_5–$CH(CH_3)$–	–Cl	0.25	2.0	0.66
	–Br	0.08	5.0	1.07
Dialkyl sulfates				
CH_3–	$-OSO_3CH_3$	0.12	99	4.34
CH_3CH_2–	$-OSO_3C_2H_5$	2.67	68	2.67
Methanesulfonates				
CH_3–	$-OSO_2CH_3$	3.25	98	4.04
CH_3CH_2–	$-OSO_2CH_3$	50.0	63	2.58
$CH_3(CH_2)_4$–	$-OSO_2CH_3$	94.0	53	2.40
$(CH_3)_2CH$–	$-OSO_2CH_3$	35.0	6.5	1.19
Tosylates				
C_6H_5–CH_2–	$-OSO_2C_6H_4CH_3$	0.12	74	2.80
p-CH_3–C_6H_4–CH_2–	$-OSO_2C_6H_4CH_3$	0.08	22	1.80
p-NO_2–C_6H_4–CH_2–	$-OSO_2C_6H_4CH_3$	1.2	99	4.34
Trialkyl phosphates				
CH_3–	$-OPO(OCH_3)_2$	200.0	53	2.40
CH_3CH_2–	$-OPO(OC_2H_5)_2$	55.0	6.9	1.22
N-Alkyl-*N*-nitrosoureas				
CH_3–	$-N(NO)CONH_2$	0.73	3.8	0.94
CH_3CH_2–	$-N(NO)CONH_2$	4.3	0.20	−0.35
$CH_3CH_2CH_2$–	$-N(NO)CONH_2$	11.0	0.24	−0.27
$CH_3(CH_2)_3$–	$-N(NO)CONH_2$	5.0	0.16	−0.45
$CH_3(CH_2)_4$–	$-N(NO)CONH_2$	3.0	0.12	−0.57
$(CH_3)_2CH$–	$-N(NO)CONH_2$	8.0	0.12	−0.57
C_6H_5–CH_2–	$-N(NO)CONH_2$	2.3	0.47	0.02
N-Alkyl-*N'*-nitro-*N*-nitrosoguanidines				
CH_3–	$-N(NO)C(NH)NHNO_2$	70.0	2.3	0.72
CH_3CH_2–	$-N(NO)C(NH)NHNO_2$	[d]	(0.1)[d]	(−0.65)[d]

a) The listed values are rough measures of the real half-lives especially for the compounds with half-lives longer than 10 h because of gradual decomposition of the alkylated NBP's, and also for those with half-lives shorter than 10 min because of experimental inaccuracy.

b) N % is the molar fraction (precentage) of the alkylated NBP produced at the end of the alkylation reaction.

c) $S_{NBP}=\log\{[H_2O]/[NBP]\times N\ \%/(100-N\ \%)\}$. Under the experimental conditions employed here, $[H_2O]=22.2$ and $[NBP]=0.10$, $S_{NBP}=\log[222\times N\ \%/(100-N\ \%)]$.

d) The half-life was so large that a reproducible value could not be obtained. Therefore, N% may contain an appreciable error.

Table III. Dependence of $N\%$ and S_{NBP} on NBP Concentration

Alkylating agent	NBP concentration (M)	N %	S_{NBP}
C_2H_5–$OSO_3C_2H_5$	0.100	68	2.67
	0.025	32	2.62
$C_6H_5CH_2$–$OSO_2C_6H_4CH_3$	0.100	74	2.80
	0.050	56	2.75
	0.025	38	2.74
$CH_3CONHC_6H_4CH_2$–Br	0.100	24	1.85
	0.025	7	1.83

indicates that the nucleophile, besides NBP, reacting with the alkylating agent may be H_2O (probably also phosphate ions to a smaller extent) but not OH^-. It should, however, be noted that exceptional cases where $N\%$ is dependent on the pH were found with some benzyl derivatives. Therefore, the composition, including pH, of the buffer in the reaction mixture should not be changed from the standard procedure described.

Molar Extinction Coefficients of the Colored Materials produced from Alkylated NBP's and Their Dependence on the Solvent used for Absorption Measurement

The λ_{max} and the molar extinction coefficient (ε) of the colored materials produced from alkylated NBP's were examined under the standard analytical conditions; the test compounds were three alkyl, one allyl, and eight benzyl derivatives. The absorption of each colored material appeared as a broad hump in the visible region, λ_{max} ranging from 520 to 535 nm, as shown in Table IV. The ε values fall in a narrow range from 29800 to 32000. A slight increase in ε is produced when an alkyl is replaced with an allyl or benzyl group. As far as the compounds examined are concerned, ε values for saturated alkylated NBP's are approximately 29900 and those for allylated and benzylated NBP's are approximately 32000. Although the accurate value of ε should be used with each alkylating agent for calculation of the molar fraction of N-alkylation, it seems to be possible to use the values of 29900 and 32000 for alkylations with saturated alkyls and with allyls and benzyls, respectively, with an error within a few percent.

Table IV. Absorption Maxima and Molar Extinction Coefficients of the Colored Materials produced from Alkylated NBP's

Alkylated NBP	Colored material	
	ε	λ_{max} (nm)
Methyl-NBP iodide	29900	534
Ethyl-NBP bromide	29800	534
n-Pentyl-NBP iodide	29800	534
Allyl-NBP bromide	31500	528
Benzyl-NBP bromide	32100	526
p-Methylbenzyl-NBP bromide	31700	529
p-Methoxybenzyl-NBP bromide	32400	533
p-Phenylbenzyl-NBP bromide	33000	526
p-Chlorobenzyl-NBP bromide	32000	518
p-Nitrobenzyl-NBP bromide	31200	512
α-Methyl-*p*-nitrobenzyl-NBP bromide	32000	536

Next, the dependence of ε on the composition of the solvent used for absorption measurement was examined. The solvent composition of the benzene layer obtained by extraction of the colored material in the standard analytical procedure is 13.8 parts of benzene and 1 part

of acetone (93%). The ε value of the benzene extract is almost independent of the solvent composition from 97 to 83% benzene as shown in Table V. It is worth noting that a large excess of acetone produced an abnormal increase in the OD value. It is considered that this increase in OD is due to the reaction of the colored material with acetone catalyzed by KOH.

Stability of the Alkylated NBP's in the Reaction Mixture

It seems that air in the reaction tube promotes the degradation of the alkylated NBP once produced. Thus, when pentyl-NBP was heated in the reaction medium sealed in a tube at 100°C, it was readily decomposed compared with the reaction which proceeded at 100°C under argon or nitrogen in the tube, as shown in Fig. 2. However, only a negligible effect of air was observed on the reaction below 60°C, so that replacement of the air in the tube with argon or nitrogen may not be essential for accurate analysis.

Alkylated NBP is definitely sensitive to the reaction temperature. The stability of pentyl-NBP in the reaction medium was examined at various temperatures. The results indicate that

TABLE V. Dependence of Optical Density of the Benzene Extract on the Solvent Composition

Solvent composition[a] benzene/acetone (v/v)	Optical density[b]
28.6	1.19
26	1.24
20	1.27
16	1.23
13.8[c]	1.22
9	1.24
5	1.30
1	12.5
0	39.9

a) Composition of the solvent for extraction of the colored material.
b) The OD values at the absorption maxima around 530 nm of the colored materials produced from an appropriate amount of *n*-pentyl-NBP.
c) The solvent composition of the extract obtained under the standard analytical conditions described in Experimental.

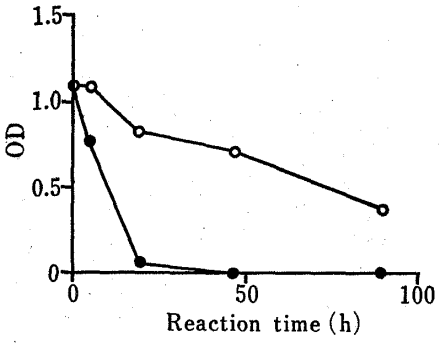

Fig. 2. Time Course of Decreases at 100°C in the OD of the Benzene Extract obtained from the Analytical Medium containing an Appropriate Amount of Pentyl-NBP under an Argon Atmosphere (—○—) and under Air (—●—) in Sealed Tubes

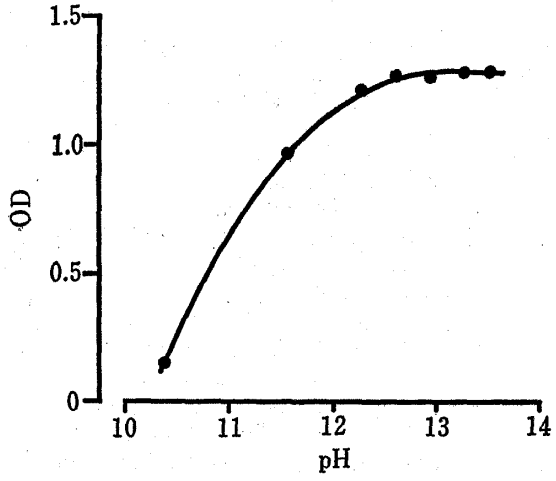

Fig. 3. Dependence of the OD of the Benzene Extract on the pH of the Reaction Mixture, containing an Appropriate Amount of Methyl-NBP, made Alkaline with NaOH for Color Development

the analysis should be carried out at 37°C and that the temperature may be raised to around 60°C only in cases where the alkylation goes too slowly at lower temperatures. It was experimentally confirmed that the values of $N\%/(100-N\%)$ were not appreciably dependent on the reaction temperature below 60°C, unless appreciable degradation of the product took place.

Extraction of the Colored Material and Determination of Its Stability

The alkylated NBP's are readily converted to neutral colored materials upon addition of caustic alkali followed by extraction with an organic solvent such as benzene. However, the conversion of the quaternary salt to the colored material is strongly depedent on the concentration of alkali added. Thus, Fig. 3 shows plots of the intensity at 534 nm of the benzene extract from the reaction with methyl iodide *versus* the pH of the reaction mixture alkalined with aqueous NaOH. These plots indicate that the reaction mixture should be made more alkaline than 12.5 N for quantitative conversion to the colored material, *i.e.*, that the amount of NaOH solution to be added for coloring should be more than 0.5 ml of 0.5 N (Table VI). Therefore, the amount which is recommended in the standard analytical procedure is sufficient for quantitative coloring of the alkylated NBP's.

TABLE VI. Dependence of the OD of the Benzene Extract on the Concentration of NaOH Solution (0.5 ml) added to 1 ml of Reaction Mixture, containing an Appropriate Amount of Methyl-NBP, for Color Development[a)]

Concentration of NaOH added (N)	pH of the reaction mixture	OD of the benzene extract at 534 nm
1/16	10.40	0.169
1/8	11.58	0.965
1/4	12.32	1.214
1/2	12.65	1.262
1	12.98	1.254
2	13.30	1.274
5	13.53	1.279

a) 0.5 ml of aqueous NaOH at the indicated concentration was added to 1 ml of the reaction mixture containing an appropriate amount of methyl-NBP diluted with 4 ml of water for color development.

The colored material thus extracted is stable in benzene if proton-donors such as water are absent, since they are strong bases, whose pK_a's are presumed to be around 11 on the basis of the plots shown in Fig. 3. In fact, when the benzene extract was dried over neutral or weakly alkaline drying agents such as $MgSO_4$, Na_2SO_4, and K_2CO_3, the OD values of the dried extracts became much smaller than those obtained when they were dried over strongly alkaline KOH pellets. When the wet benzene extracts were filtered through filter paper to make the extract transparent, the OD values were also much diminished. The colored material might have been partly absorbed into the water phase of the filter paper as protonated forms.

It was confirmed that the colored material once extracted was stable even 1 d after the extraction, provided that the extracts once dried over KOH pellets were tightly sealed so as to prevent moisture and carbon dioxide absorption.

Discussion

This analytical method is a one-pot procedure giving the approximate ratio of the rate of alkylation of NBP to that of hydrolysis in the same solvent system. Provided that the hydrolysis follows the apparent rate formula, $\text{Rate}=k_{obs}[\text{alkylating agent}][H_2O]$, in the given reaction medium, a kinetic meaning can be given to S_{NBP}, as follows. Provided that the *N*-alkylation and hydrolysis of a given alkylating agent each follow bimolecular kinetics, S_{NBP} is the ratio of the second-order rate constants of the following reaction.

$$\text{R–L} + \text{NBP} \xrightarrow{k_{2,N}} \text{R–NBP} + \text{L}^-$$

$$\text{R–L} + \text{H}_2\text{O} \xrightarrow{k_{2,O}} \text{R–OH} + \text{HL}$$

where $k_{2,N}$ and $k_{2,O}$ are rate constants for the alkylation and the hydrolysis, respectively, and L is the leaving group of the alkylating agent.
Then,

$$d(\text{R–NBP})/dt = k_{2,N}[\text{R–L}][\text{NBP}]$$

$$d(\text{R–OH})/dt = k_{2,O}[\text{R–L}][\text{H}_2\text{O}]$$

In cases where [NBP] and $[\text{H}_2\text{O}] \gg [\text{R–L}]$,

$$\frac{d(\text{R–NBP})}{dt} / \frac{d(\text{R–OH})}{dt} = N\%/(100-N\%)$$

where $N\%$ is the molar percent ratio of R–NBP produced to all the other reaction products at the end point of the reaction.
Therefore,

$$N\%/(100-N\%) = k_{2,N}[\text{NBP}]/k_{2,O}[\text{H}_2\text{O}]$$

$$S_{\text{NBP}} = \log(k_{2,N}/k_{2,O})$$

Alternatively, in cases where the reactions of a given alkylating agent with NBP and H_2O each follow unimolecular kinetics, S_{NBP} is the competition factor (f)[14,15] of the carbonium ion produced from the alkylating agent.

$$\text{R–L} \xrightarrow{k_1} \text{R}^+$$

$$\text{R}^+ + \text{NBP} \xrightarrow{k'_N} \text{R–NBP}$$

$$\text{R}^+ + \text{H}_2\text{O} \xrightarrow{k'_O} \text{R–OH}$$

Then,

$$N\%/(100-N\%) = k'_N[\text{NBP}]/k'_O[\text{H}_2\text{O}]$$

$$S_{\text{NBP}} = \log(k'_N/k'_O) = \log f$$

Some of the compounds studied here, such as benzyl halides, and methyl methanesulfonate, are typical S_N2 type electrophiles[16,17] and some others such as neopentyl methanesulfonate are S_N1 type.[14] With regard to nitrosoureas, the following mechanism may be involved.[9]

$$\text{R–N(NO)–CO–NH}_2 + \text{H}_2\text{O} \xrightarrow[\text{slow}]{} \text{R–N=N–OH}$$

$$\text{R–N=N–OH} + \text{NBP} \xrightarrow[\text{fast}]{} \text{R–NBP}$$

$$\text{R–N=N–OH} + \text{H}_2\text{O} \xrightarrow[\text{fast}]{} \text{R–OH}$$

Thus, the S_{NBP} of nitrosoureas represents the competition factor of the carbonium ions produced, although the degradations of the nitrosoureas follow S_N2 kinetics. Nitrosoguanidines may belong to the same category of reactants as nitrosoureas.[19]

Correlation of S_{NBP} with the Substrate Constant, s, of the Swain–Scott Equation

It is considered that S_{NBP} is significantly related to the substrate constant, s, in the Swain–Scott equation.[8,21]

$$\log(k_Y/k_W) = sn$$

where k_Y and k_W are the second-order rate constants for the reactions with nucleophile Y and with water, respectively, and n is the nucleophilic constant which expresses the relative

nucleophilic strength of a given nucleophile with respect to that of H_2O, and s is the substrate constant which expresses the relative sensitivity of a given alkylating agent to changes in the nucleophilicity of the nucleophiles relative to CH_3Br. The S_{NBP} values of some of the compounds studied here were correlated with their s values given in the literature.[2,8,14,19] A linear correlation was found for almost all the compounds examined, except for isopropyl methanesulfonate, the regression equation for the correlation being given below.

$$s = 0.123(\pm 0.0078)S_{NBP} + 0.318(\pm 0.019)$$
$$\text{correlation coefficient} = 0.997$$

The number of samples is 10 and the 95% confidence limit is given in parentheses in the equation. Data are given in Table VII and Fig. 4. The deviation from the linear correlation found with isopropyl methanesulfonate may reflect a field or medium effect on the chemoselectivity of this methanesulfonate. The chemoselectivities of the samples fitting the regression equation are insensitive but that of isopropyl methanesulfonate may be sensitive to the difference in the composition of the solvents used in obtaining the constants, S_{NBP} and s. It may be speculated that typical S_N2 and S_N1 types of alkylating agents are insensitive to changes in the reaction medium but that intermediate agents, the molecularity of which depends strongly on the nature of the solvent, are sensitive, thus leading to the differences between S_{NBP} and s. These results suggest that quantitative correlation of biological activity with chemical properties, such as the chemoselectivity and reaction rate, should perhaps be made with thorough consideration of the difference between the experimental solvent system and the environment around the physiological target molecules such as cellular DNA.[21,22]

Table VII. Correlation of S_{NBP} with Substrate Constant, s, at 37°C

Compound	s[a]	S_{NBP}
N-Methylnitrosourea	0.42(19)	0.94
N-Ethylnitrosourea	0.26(19)	−0.35
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	0.42(19)	0.72
N-Ethyl-*N'*-nitro-*N*-nitrosoguanidine	0.26(19)	−0.65
Methyl methanesulfonate	0.83(14)	4.04
Ethyl methanesulfonate	0.64(14)	2.58
Isopropyl methanesulfonate	0.29(14)	1.19
Neopentyl methanesulfonate	0.45(14)	1.08
Ethyl tosylate	0.66(8)	3.04
Dimethyl sulfate	0.86(14)	4.34
Diethyl sulfate	0.64(14)	2.67

a) The number in parentheses is the reference number.

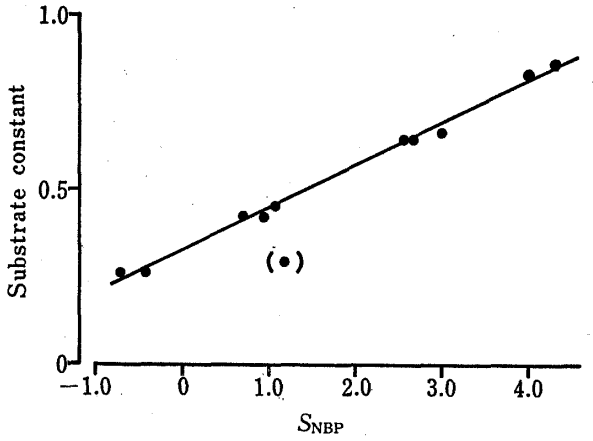

Fig. 4. Plots of the Substrate Constants reported in the Literature *versus* S_{NBP} Values obtained in This Study

The line was drawn by regression analysis with ten compounds examined. Isopropyl methanesulfonate, indicated by parentheses in the figure, is excluded from the analysis.

Recently, Bartsch *et al.* reported[2] that the substrate constant was well correlated with the relative rate of alkylation of NBP with respect to that of hydrolysis, where the rates were measured in separate experiments, and suggested that s seemed to be a quantitative measure of carcinogenicity among some classes of carcinogens. The present method for the characterization of alkylating ability has the practical advantage of experimental simplicity in the characterization of a wide variety of alkylating agents. It is noteworthy that quantitative treatments of NBP alkylations have recently been attempted in connection with biological alkylating agents.[23,24]

The S_{NBP} values obtained in the present study are of interest in relation to the chemical structures, as will be fully discussed in a forthcoming paper in connection with the hard and soft acids and bases principle, in addition to the substrate constant.

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