[Chem. Pharm. Bull.
34(10)4103—4108(1986)]

Studies on Chemical Carcinogens and Mutagens. XL.[1] Chemoselectivity of Acylating Agents toward Aniline in Aqueous Medium

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(Received April 17, 1986)

The chemoselectivities of 24 acylating and carbamoylating agents were evaluated in terms of the relative rate of aniline acylation *versus* the rate of hydrolysis in dioxane–phosphate buffer (pH 6). The chemoselectivity constant was defined as the logarithm of $N[H_2O]/(1-N)$ [aniline], where N is the molar fraction of the acylating agent consumed for the acylation of aniline, hence $(1-N)$ is that for the hydrolysis. From these constants, the relative rates of nucleophilic attack of these acylating agents on aniline and water are estimated to range from 2 to 540000. These constants are linearly correlated with the substrate constants defined by Swain and Scott. Some structure–chemoselectivity relations are discussed.

Keywords——chemoselectivity; acylation; carbamoylation; substrate constant

Acylating agents are a common class of electrophiles and are widely used for derivatization or protection of protic functional groups. Biologically, very few acylating agents are known to be active in mutagenesis and/or carcinogenesis,[2] although they are chemically as reactive (even under physiological conditions) as the genotoxic electrophiles such as alkylating agents and nitrenium ion-producing aromatic hydroxylamines.[3] It is suspected that their biological inertness is due to their chemical characteristics which allow them to be effectively scavenged by nucleophilic biomolecules in the cytoplasm, so that they are not capable of producing genotoxic modifications of the target deoxyribonucleic acid in the cell nucleus. In this connection, the chemoselectivity has been discussed for a limited number of acylating agents,[4,5] but no systematic surveys have been reported. The present study was designed to evaluate quantitatively the chemoselectivity of various types of acylating and carbamoylating agents in terms of the relative rate of aniline acylation *versus* the rate of hydrolysis in an aqueous buffer containing aniline. The evaluated chemoselectivity constants are linearly correlated with the substrate constants (s) defined by Swain and Scott.[6] A brief discussion is included on the relation between the substrate constant and the genotoxicity.

Experimental

Materials——Aniline, benzanilide, acetanilide, and all acylating agents were purchased from Tokyo Kasei Kogyo Co. (Tokyo), Wako Pure Chemicals Ltd. (Osaka), or Nakarai Chemical Industries Ltd. (Kyoto). The other acylated anilines used as references for chromatographic analyses were prepared in our laboratory. The melting points were measured with a micro melting point apparatus (Yanaco) and those in parentheses are taken from Beilsteins Handbuch der Organischen Chemie: *p*-methoxybenzanilide, mp 177 (171) °C; *p*-methylbenzanilide, 152 (145) °C; *p*-chlorobenzanilide, 206 (194) °C; *p*-nitrobenzanilide, 222 (218) °C; *p*-methoxybenzenesulfonanilide, 111 (111) °C; *p*-methylbenzenesulfonanilide, 103 (103) °C; benzenesulfonanilide, 114 (113) °C; *p*-chlorobenzenesulfonanilide, 110 (104) °C; *p*-nitrobenzenesulfonanilide, 173 (171) °C; 2,6-dichlorobenzanilide, 179 °C; mesitylenesulfonanilide, 116 (114) °C; trifluoroacetanilide, 92 (88) °C; *N*,*N*-dimethyl-*N*′-phenylurea, 136 (134) °C; *N*,*N*-diethyl-

N'-phenylurea, 91 (90) °C; triphenylurea, 142 (136) °C; N-ethyl-N'-phenylurea, 104 (102) °C; N,N'-diphenylurea, 251 (247) °C; phenyl N-phenylcarbamate, 131 (126) °C. All the preparations were subjected to elementary analysis and checked for purity by high performance liquid chromatography (HPLC) and ^{1}H-nuclear magnetic resonance (NMR) spectroscopy. All the alkylating agents used were our stock preparations.[7]

Determination of Relative Rate of Acylation of Aniline *versus* Hydrolysis of Acylating Agents in Dioxane–Phosphate Buffer (1 : 2, v/v)——The acylated aniline produced in the reaction medium was quantitatively analyzed at appropriate intervals until the value became constant. The procedures are as follows.

Procedure A——An acylating agent (0.1 mmol) dissolved in 5 ml of dioxane was added to a mixture of 5.0 mmol of aniline in 5 ml of dioxane and 20 ml of 0.2 M phosphate buffer (pH 6.0) at 37 °C. At appropriate intervals, 4 ml aliquots of the reaction mixture were successively sampled and each aliquot was combined with 8 ml of H_2O and extracted with 6 ml of $CHCl_3$. The extract was washed with 10 ml of 1 N HCl and dried over anhydrous $MgSO_4$, and quantitatively analyzed by HPLC on a JASCO Finepack SIL 4.6 mm × 250 mm column using a JASCO TWINCLE apparatus equipped with a UVIDEC-100-III ultraviolet detector adjusted to 254 nm wavelength. The eluting solvent was $CHCl_3$. Quantitation was done by reference to working curves previously prepared.

Procedure B——A smaller amount of acylating agent (5 μmol) in 5 ml of dioxane and 50 μmol of aniline in 5 ml of dioxane were combined with 20 ml of 0.2 M phosphate buffer (pH 6.0) and worked up as described in procedure A.

Procedure C——A smaller amount of acylating agent (0.5 μmol) in 5 ml of dioxane and 5 μmol of aniline in 5 ml of dioxane were combined with 20 ml of the same buffer and worked up as described in procedure A.

Determination of Relative Rate of Alkylation of Aniline *versus* Hydrolysis of Alkylating Agents in Dioxane–Phosphate Buffer (1 : 2, v/v)——All the procedures including chromatographic analysis were the same as those described in procedure A for acylating agents. Some of the analyses were performed by gas-chromatography (GC) on a Celite 545 paraffin wax (5%) plus KOH (2%) 3.2 mm × 2 m column using a Shimadzu GC8A apparatus equipped with an hydrogen flame ionization detector. It was confirmed with each alkylating agent that only the monoalkylated product was detected on a GC chart.

Definition of S_A, a Measure of Chemoselectivity toward Nucleophiles——The disappearance rate of a given acylating agent in phosphate buffer containing an appropriate amount of aniline can be written as follows.

$$d[\text{acylating agent}]/dt = \{k_A[\text{aniline}] + k_h\}[\text{acylating agent}]$$

where k_A is the second order rate constant of the aniline acylation and k_h is the observed rate constant of hydrolysis of the acylating agent. When the reaction proceeds with a large excess of aniline compared with the amount of acylating agent, the ratio of k_A to k_h is given simply by:

$$k_A/k_h = \frac{N}{(1-N)[\text{aniline}]}$$

where N is a molar fraction of the acylating agent consumed for the acylation of aniline, and hence $(1-N)$ is that for the hydrolysis. This ratio can be used as a quantitative measure for the relative chemoselectivity of acylating agents toward the NH_2 group of aniline in relation to the hydrolysis in a given reaction medium. In the present study, the logarithm of this ratio multiplied by the concentration of water, $[H_2O]$, in the reaction medium is defined as S_A:

$$S_A = \log \frac{N}{(1-N)} \frac{[H_2O]}{[\text{aniline}]} \qquad (1)$$

It is worth noting that since the observed rate constant for hydrolysis is given by $\{k_{H_2O}[H_2O] + k_{OH}[OH^-] + k_H[H_3O^+] + K_0[\text{others}]\}$, S_A is not equal to $\log k_A/k_{H_2O}$. However, on the assumptions that, in the medium at pH 6.0, $k_{H_2O}[H_2O]$ may be the predominant term and that the solvent effect on the relative rate ratio between k_A and k_{H_2O} may not be appreciable, then S_A may be regarded as a rough (but not a strict) measure of the ratio of the second-order rate constant for electrophilic attack on the nitrogen of aniline *versus* that for attack on the oxygen of water. In cases where the acylation proceeds by first-order kinetics, S_A may approximate to the logarithmic competition factor of electrophilic attacks of an acyl cation on aniline and water. Since a reaction mixture prepared with 20 ml of the phosphate buffer and 10 ml of dioxane has a volume of 29.7 ml, the molar concentration of H_2O is 37.4 M, and those of aniline are 168, 1.68, and 0.168 mM in procedures A, B, and C, respectively.

S_A Values Obtained from Reactions with Various Concentrations of Aniline——In cases where the experimentally determined N value becomes close to unity, the term $N/(1-N)$ in Eq. 1 may contain large error; therefore, the concentration of aniline must be lowered. Thus, it was confirmed with four acylating agents (**2**—**4**, **8**) that the S_A value was independent of the concentration of aniline involved in the reaction medium. The concentration of acylating agent used was less than 1/10 molar equivalent with respect to aniline. The results are shown in Table I.

Results

As shown in Table II, 7 carboxylic acid chlorides (**1**, **2**, **4**—**8**), 1 carboxylic acid bromide

TABLE I. Dependence of S_A on Concentration of Aniline[a)]

Acylating agent	(μmol)	Aniline (μmol)	N	S_A
Benzoyl chloride (**2**)	5.0	50	0.94	5.54
	2.5	25	0.89	5.56
	1.0	10	0.73	5.48
	0.5	5	0.61	5.54
Benzoyl bromide (**3**)	2.5	25	0.61	4.84
	1.0	10	0.39	4.85
	0.5	5	0.24	4.83
p-CH_3O–benzoyl chloride (**4**)	5.0	500	0.62	3.57
	5.0	250	0.38	3.44
	5.0	100	0.24	3.54
	5.0	50	0.12	3.48
p-NO_2–benzoyl chloride (**8**)	5.0	50	0.96	5.73
	0.5	5	0.67	5.65

a) The reaction medium consisted of a mixture of 10 ml of dioxane and 20 ml of 0.2 M phosphate buffer (pH 6.0).

TABLE II. Chemoselectivity of Some Acylating and Carbamoylating Agents

Compound	Procedure	N	S_A	s[a)]
Carboxylic acid halides				
Acetyl chloride (**1**)	B	0.08	3.29	0.79
Benzoyl chloride (**2**)	C	0.61	5.54	1.44
Benzoyl bromide (**3**)	B	0.74	4.80	1.23
p-CH_3O–benzoyl chloride (**4**)	B	0.12	3.48	0.85
p-CH_3–benzoyl chloride (**5**)	B	0.64	4.60	1.17
p-Cl–benzoyl chloride (**6**)	B	0.96	5.73	1.50
2,6-Di-Cl–benzoyl chloride (**7**)	B	0.27	3.91	0.97
p-NO_2–benzoyl chloride (**8**)	C	0.67	5.65	1.48
Sulfonic acid chlorides				
Benzenesulfonyl chloride (**9**)	B	0.60	4.52	1.15
p-CH_3O–benzenesulfonyl chloride (**10**)	B	0.45	4.26	1.07
p-CH_3–benzenesulfonyl chloride (**11**)	B	0.68	4.67	1.19
Mesitylenesulfonyl chloride (**12**)	B	0.37	4.12	1.03
p-Cl–benzenesulfonyl chloride (**13**)	B	0.60	4.52	1.15
p-NO_2–benzenesulfonyl chloride (**14**)	B	0.53	4.40	1.11
Carboxylic acid anhydrides				
Acetic anhydride (**15**)	A	0.60	2.52	0.57
Trifluoroacetic anhydride (**16**)	A	0.50	2.35	0.52
Benzoic anhydride (**17**)	A	0.30	1.98	0.41
Carbamoyl chlorides				
Dimethylcarbamoyl chloride (**18**)	A	0.0095	0.33	−0.07
Diethylcarbamoyl chloride (**19**)	A	0.0175	0.60	0.01
Diphenylcarbamoyl chloride (**20**)	B	0.20	3.74	0.92
Isocyanates				
Ether isocyanate (**21**)	B	0.18	3.69	0.91
Phenyl isocyanate (**22**)	B	0.41	4.19	1.05
Chloroformate				
Phenyl chloroformate (**23**)	A	0.92	3.41	0.83
Active ester				
p-NO_2–phenyl acetate (**24**)	A	0.88	3.21	0.77

a) Swain–Scott's substrate constant estimated from S_A.

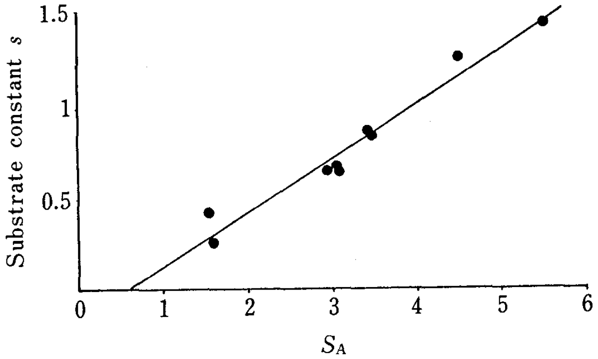

Fig. 1. Correlation of S_A Values with Swain–Scott's Substrate Constants (s)
$s = 0.290S_A - 0.163$ ($r = 0.979$; sample number, 9).

(**3**), 6 sulfonic acid chlorides (**9**—**14**), 3 carboxylic acid anhydrides (**15**—**17**), 3 carbamic acid chlorides (**18**—**20**), 2 isocyanates (**21**, **22**), 1 chloroformate (**23**) and 1 active ester (**24**) were subjected to chemoselectivity analysis by using the aniline-buffer system described in Experimental. The results are shown in Table II, each N value being the average of more than three measurements. The deviations from the average fell within ±0.05 wherever N is not more than 0.90. The observed S_A values range widely from 0.33 to 5.73, depending on the structure of both the acyl moiety and the leaving group. In other words, the relative acylation rate of the nitrogen on aniline *versus* the rate of hydrolysis ranges from 2.1 to 540000.

In order to correlate the S_A value with the substrate constant (s) defined by Swain and Scott,$^{6)}$ 7 alkylating agents and 2 acylating agents, the s values of which have already been reported, were selected for the present chemoselectivity analysis. The regression analysis of these results gave the linear regression equation shown in Fig. 1.$^{8)}$ The s values of all the acylating agents examined were evaluated by using this equation, and are listed in Table II. The s values of the alkylating agents reported in the literature range from 0.2 to 2.0$^{6,7,9)}$ and those of the acylating agents found in the present study range from -0.067 to 1.50, as seen in Table II.

Discussion

Dimethyl- and diethylcarbamoyl chlorides show the lowest chemoselectivity among all the acylating and alkylating agents examined. Acid anhydrides show medium values of chemoselectivity, comparable to those of alkylating agents. All other acylating agents show higher chemoselectivities. Some structural dependences of the S_A value were found as follows.

Lowest Chemoselectivity of Dialkylcarbamoyl Chlorides

Dimethyl- and diethylcarbamoyl chlorides (**18**, **19**) showed extraordinarily low chemoselectivities, 0.33 and 0.60, respectively. They are known to undergo carbamoylation through the S_N1 mechanism$^{4)}$; the lone-pair electrons on the nitrogen must assist in acylium ion stabilization so as to facilitate unimolecular heterolysis of the carbonyl-chlorine bond. It is worth noting that these two carbamoylating agents are known to be carcinogenic.$^{2)}$ Diphenylcarbamoyl chloride (**20**), where the N-lone pair is delocalized over two benzene rings, showed a much higher chemoselectivity value of 3.74.

Substituent Effects on S_A Values of *p*-Substituted Benzoyl and Benzenesulfonyl Chlorides

The S_A values of 5 benzoyl chlorides are plotted *versus* the σ_p^+ of the substitutent in Fig. 2. The S_A tends to increase proportionally with decrease in the electron-donating nature of the substitutent and reaches a plateau at the positive ordinate, as shown in Fig. 2. One explanation might be that the electron-donating nature of the substituent may facilitate an S_N1-like acylation through stabilization of the acylium ion, R–CO$^+$, *i.e.*, the acylation becomes less chemoselective. Acylations with rather electron-deficient benzoyl derivatives

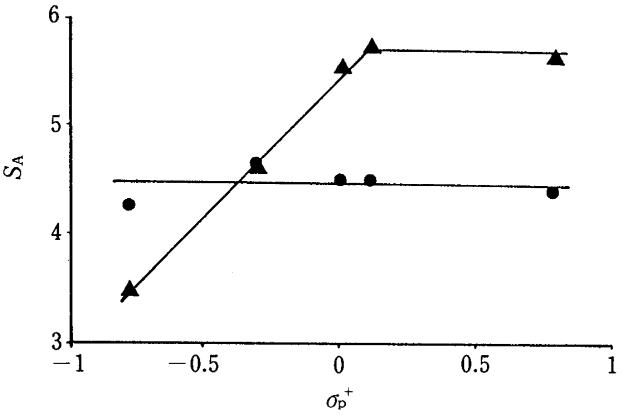

Fig. 2. Correlation of S_A Values with σ_p^+ of *p*-Substituted Benzoyl Chlorides (—▲—) and *p*-Substituted Benzenesulfonyl Chlorides (—●—)

(**6**—**8**) might all proceed in a purely bimolecular process; the S_A values appear to be independent of the nature of the substituent. This explanation seems to be supported by the data on benzenesulfonyl chlorides. Thus, the S_A values of 5 *p*-substituted benzenesulfonyl chlorides fell in a narrow range, independently of the electronic nature of the substituent, as shown in Fig. 2. The sulfonyl group itself is more electron-withdrawing than the carbonyl group, so that the sulfonyl chlorides may all react in bimolecular processes.

The difference in S_A between sulfonyl chlorides (4.2—4.7) and electron-deficient carbonyl chlorides (5.5—5.7) may be due to the difference in their electronic and/or steric effects in the interaction with nucleophiles at the transition state.

leaving group	dependence of S_A on acyl moiety				
chlorides:	CH_3CO– (3.29)	<	$C_6H_5SO_2$– (4.52)	<	C_6H_5CO– (5.54)
	$(CH_3)_2NCO$– (0.33)	<	$(C_2H_5)_2NCO$– (0.60)	<	$(C_6H_5)_2NCO$– (3.74)
(isocyanates):	C_2H_5– (3.69)	<	C_6H_5– (4.19)		
(anhydrides)	C_6H_5CO– (1.98)	<	CF_3CO– (2.35)	<	CH_3CO– (2.52)
acyl moiety	**dependence of S_A on leaving group**				
benzoyl:	–$OCOC_6H_5$ (1.98)	<	–Br (4.80)	<	–Cl (5.54)
acetyl:	–$OCOCH_3$ (2.52)	<	–OC_6H_4–NO_2 (3.21)	<	–Cl (3.29)

Chart 1

Other Trends of Structural Dependence

The sets of data shown in Chart 1 may be of interest for comparison.

The present study has revealed in outline the chemoselectivities of some common acylating agents. A further study along this line is now being pursued on these agents in organic solvents such as benzene and pyridine.

References and Notes

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9) The correlation equation includes a constant term of -0.163 (not zero). This might be due to the facts that S_A is not an exact measure of the relative rate ratio toward aniline and water molecules and that the solvent effect is ignored. The Swain–Scott's substrate constants are those obtained from the rate ratios between nucleophiles and the water molecule itself measured in purely aqueous media.