Quantitative Evaluation of the Reactivity of Alkylating Agents

Nicholas Bodor^a, James J. Kaminski^b, S. D. Worley^c, and Steven H. Gerson^a

Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610; Schering Corporation, Bloomfield, N. J. 07003; and Department of Chemistry, Auburn University, Auburn, Alabama 36830

Z. Naturforsch. **35b**, 758–763 (1980); received February 4, 1980

Alkylating Agents, Soft Drugs, NMR Spectra, Competitive Alkylation, Soft Quaternary Salts

A sensitive and reproducible method for quantitative evaluation of the relative reactivities of alkylating agents was developed, based on competitive alkylation. The method is superior to the known colorimetric methods. The reactivities of the agents could also be correlated with the ¹³C chemical shifts of the α -methylene. The method was successfully applied for the ranking of "soft" alkylating agents of low reactivity.

It was recently found that "soft" alkylating agents of the type $\text{R}-\overset{\text{R}_1}{\underset{|}{\text{COOCH}}}-\text{Y}$ (**1**) (Y = Cl or Br) have anticancer activity [1]. (Soft drugs are defined as active therapeutic agents which undergo predictable and controllable metabolism after they achieve their therapeutic role [2]. Thus, "soft" alkylating agents of the type **1** are subject to ester cleavage (enzymic or chemical), according to the equation:

The "soft" terms, thus reflect the easily biodegradable characteristics of these compounds, opposite to the conventional "hard" (non-biodegradable) alkylating agents.)

In order to establish a relation between the activity and structure of these agents, a method to evaluate quantitatively their relative alkylating reactivities was developed, which is compared to the method of Epstein *et al.* [3]. The Epstein procedure is based on the spectrophotometric analysis of the colored product **2** formed from 4-(4-nitrobenzyl)pyridine (NBP) and alkylating (or arylating agent) (A-X), by reacting them in methyl ethyl ketone, followed by addition of a base (deprotonation of the benzylic methylene):

An alternate method [4] used acetophenone as a solvent and 180° as the reaction temperature, and a large number of alkylating agents were thus assayed, including N-mustards [5].

It is clear that NBP is a weak base. Thus, under the conditions suggested [3], no reaction was observed with the soft alkylating agents (**1**) having also very low reactivity. It was found that the expected colored products can be obtained if **1** is heated at 70 °C for one hour with a large excess of the neat NBP. Using different amounts of **1**, a plot of the concentration *vs* absorbance of **2** resulted in straight lines of different slopes, apparently depending on the reactivity of **1**. For characterization of the alkylating reactivities, the M 301 values suggested by Epstein [3] were determined (M 301 is the moles of the alkylating agent required to produce a colored solution having 0.301 absorbance at 565 nm). The values obtained for a series of closely related soft alkylating agents are listed in Table I. It can be seen that although methyl iodide was predicted to be more reactive than **1a–1h**, the structurally related compounds cannot be differentiated with sufficient accuracy. It is difficult to believe that the bromo derivative **1h** is less reactive than **1a**.

The drastic conditions of the alternate method [4] were not investigated. It is difficult to expect that closely related compounds will show reliable differences by reacting them at 180 °C for 3.5 min [4]. It was also found that the intensity of the color of **2** is time dependent, contributing to the inaccuracy.

A new method [1] for quantitative evaluation of relative alkylating reactivities was then investigated in detail. The method is based on a competitive alkylation of a selected tertiary amine (for example, 3-acetoxy-quinuclidine (**3**)) by a 1:1 molar mixture

^a University of Florida, ^b Schering Corporation, ^c Auburn University.

* Reprint requests to Dr. N. Bodor.
0340-5087/80/0600-0758/\$ 01.00/0

Table I. Moles of alkylating agents (1) required to produce colored solution of 2 having 0.301 absorbance ($R_1 = H$ in all cases).

Nr. ^a	R	Y	M 301 ($M \times 10^6$) ^b
1a	(CH ₃) ₃ C	Cl	0.826 ± 0.05
1b	CH ₃ (CH ₂) ₂	Cl	0.890 ± 0.05
1c	CH ₃ (CH ₂) ₄	Cl	1.05 ± 0.05
1d	CH ₃ (CH ₂) ₁₀	Cl	1.43 ± 0.05
1e	CH ₃ (CH ₂) ₁₂	Cl	1.07 ± 0.05
1f	CH ₃ (CH ₂) ₁₄	Cl	1.17 ± 0.05
1g	C ₆ H ₅	Cl	0.998 ± 0.05
1h	C ₆ H ₅	Br	1.06 ± 0.05
Methyl iodide ^c			0.714 ± 0.05

^a 1a is commercially available, compounds 1b–1h were synthesized previously [1]. The benzoates 1g and 1h were obtained according to ref. [8].

^b An accuracy of 5 percent [3] was considered.

^c Methyl iodide was included for comparison.

of the compound studied and a standard alkylating agent, followed by the direct analysis of the composition of the reaction mixture:

The tertiary amine 3 was chosen because of its structural rigidity, while the easily available chloromethyl pivalate (1a) was selected as the standard agent. Using a 1:1:1 molar mixture of 1:1a:3, the ratio of the amounts of the products 4/5 quantitatively reflects the relative alkylating reactivity (RAR).

The present work reports studies on the sensitivity of the competitive method, by comparing reactivities of the structurally closely related series of substituted benzoylmethyl halides:

Nr.	X	Y
1g	H	Cl
1h	H	Br
1i	<i>p</i> -CH ₃ O	Cl
1j	<i>p</i> -CH ₃	Cl
1k	<i>m</i> -Br	Cl
1l	<i>m</i> -NO ₂	Cl
1m	<i>p</i> -Cl	Cl
1n	<i>p</i> -Br	Cl
1o	<i>p</i> -NO ₂	Cl

The reaction mixtures of 4g–4o vs 5 were analyzed by NMR, comparing the sharp singlet resonance

signals corresponding to the $\geq N^+-CH_2-O-\overset{O}{\parallel}C-R$ methylene, at expanded sweep width. The ¹H and ¹³C chemical shifts of the methylene group in 1a–1o and the ¹H shifts of the methylene protons in the corresponding soft quaternary salts 4 are shown in Table II.

Table II. ¹H and ¹³C chemical shifts of the methylene group of selected chloromethyl carboxylates^a (3) and the corresponding soft quaternary salts^b (4).

Compound Nr.	Chemical shift in 1 δ^1H [ppm]	Chemical shift in 4 $\delta^{13}C$ [ppm]	Chemical shift in 4 δ^1H [ppm]
a	5.70		5.23(5) ^c
g	5.97	69.40	5.47
i	5.90	69.38	5.50
j	5.90	69.33	5.50
k	6.00	69.44	5.57
l	6.03	69.69	5.63
m	5.93	69.41	5.57
n	5.93	69.35	5.57
o	6.00	69.63	5.70

^a Chemical shifts measured in CDCl₃ solutions.

^b Chemical shifts measured in D₂O-d⁶-acetone.

^c Chloromethyl pivalate (1a) and the corresponding salt 5 included for comparison.

It can be seen that the ¹H shifts are not too sensitive to the ring substituents, but the ¹³C shifts show the expected trend.

The ¹H chemical shifts of 4g–4o are sufficiently separated from that of 5, which was selected as the standard. The results of the competitive alkylation are shown in Table III.

The percent product composition values are the average of two separate experiments found to be reproducible with ±0.5 percent. Taking the reactivity of the unsubstituted 1g as unity, the RAR values were normalized and listed as pX/pH. Plotting pX/pH values against the corresponding substituent constants (σ°) [6] a good correlation was obtained, as shown in Fig. 1.

Benzyl chloride (6) and the unhindered soft alkylating agent chloromethyl hexanoate (7) were also included in Table III, for comparison. As expected, 6 is much more reactive than the soft agents 1g–1o. Finally, as expected, the bromo derivative 1h is about 18 times more reactive than the corresponding chloro compound 1g.

Table III. Relative alkylating reactivities (RAR) of substituted halomethyl benzoates.

Compound	X =	Y	Product composition [%]		RAR (ratio) 4/5	PX/PH
			4	5		
1g	H	Cl	43	57	0.75	1.00
1h	H	Br	93	7	13.29	
1i	<i>p</i> -CH ₃ O	Cl	51	49	1.04	1.39
1j	<i>p</i> -CH ₃	Cl	49	51	0.96	1.28
1k	<i>m</i> -Br	Cl	38	62	0.61	0.81
1l	<i>m</i> -NO ₂	Cl	31	69	0.45	0.60
1m	<i>o</i> -Cl	Cl	36	64	0.56	0.75
1n	<i>p</i> -Br	Cl	38	62	0.61	0.81
1o	<i>p</i> -NO ₂	Cl	35	65	0.54	0.72
6	C ₆ H ₅ CH ₂ Cl		79	21	3.76	
7	CH ₃ (CH ₂) ₄ COOCH ₂ Cl		35	65	0.54

Fig. 1. Plot of relative reactivities (pX/pH) of substituted chloromethyl benzoates *vs* σ^o . Slope -0.32 ; $r = 0.90$.

The relative differences in the ^{13}C chemical shifts ($\Delta\delta^{13}\text{C} = \delta^{13}\text{C}_X - \delta^{13}\text{C}_H$) in the series **1h–1o** were also plotted against σ^o , obtaining a fairly good correlation.

In conclusion, the competitive alkylation provides a sensitive and reliable method for predicting quantitatively the alkylating reactivity of a wide variety of agents. By choosing proper standards, a full scale of reactivity of the known agents can be

Fig. 2. Plot of ^{13}C chemical shifts of the methylene carbon in substituted chloromethyl benzoates *vs* σ^o . Slope $= 0.33$; $r = 0.89$.

developed, since the method is certainly not limited to the soft alkylating agents studied. Conventional alkylating agents, N-mustards, *etc.* could quantitatively be compared by this method. As the ratio of the products formed under the conditions used (1:1:1 starting molar ratios and consuming all the amine) is not simply proportional with k_1/k_2 [7], it is possible to achieve better selectivity while using

the amine in large excess or not allowing the reaction to be completed. In a relatively short time, the ratio of the products formed is proportional with k_1/k_2 , as the starting alkylating agent's concentrations is the same. If the "short time" restriction does not hold, k_1/k_2 is proportional with the ratio of the logarithm of the products.

Experimental

1. Synthesis

3-Acetoxyquinuclidine (5) was prepared by acetylation of 3-quinuclidinol. The product was purified by vacuum distillation. M.p. 34–36 °C; b.p. 76–78 °C (0.8 mm).

Benzyl chloride (6) and chloromethyl pivalate (**1a**) were obtained from Aldrich Chemical Company.

Chloromethyl benzoate (**1h**) was prepared from benzoyl chloride and paraformaldehyde using the method described by Ulich and Adams [8], b.p. 84–85 °C (1.2 mm) [Lit. [8] b.p. 114–115 °C (8 mm)] IR (neat) 3060, 2990, 1730, 1590, 1490, 1450, 1340, 1300, 1250, 1080, 800 and 720 cm^{-1} ; PMR (CDCl_3) δ 7.0–8.2 (m, 5H) and 5.97 (s, 2H) ppm. Bromomethyl benzoate (**1g**) was obtained analogously, using benzoyl bromide, b.p. 62–65 °C (0.3 mm) [Lit. [8] 135–137 °C (19 mm)].

Using the procedure described for the preparation of **1h**, the following α -chloromethyl benzoates were prepared:

Chloromethyl p-methoxybenzoate (**1i**): b.p. 119 to 121 °C (0.5 mm), IR (neat) 2980, 2915, 1720, 1595, 1500, 1430, 1330, 1245, 1165, 1070, 845, 770 and 710 cm^{-1} ; PMR (CDCl_3) δ 6.7–8.2 (AA'BB', 4H), 5.90 (s, 2H) and 3.78 (s, 3H) ppm.

Analysis for $\text{C}_9\text{H}_9\text{ClO}_3$

Calcd C 53.88 H 4.52,
Found C 54.14 H 4.72.

Chloromethyl p-methylbenzoate (**1j**): b.p. 92–94 °C (1 mm), IR (neat) 2990, 2960, 1730, 1600, 1435, 1340, 1255, 1175, 1080, 1020, 840, 750 and 720 cm^{-1} ; PMR (CDCl_3) δ 7.0–8.1 (AA'BB', 4H), 5.90 (s, 2H) and 2.33 (s, 3H) ppm.

Analysis for $\text{C}_9\text{H}_9\text{ClO}_2$

Calcd C 58.55 H 4.91,
Found C 58.94 H 5.27.

Chloromethyl m-bromobenzoate (**1k**): b.p. 110 to 113 °C (0.8 mm), IR (neat) 3030, 2990, 1730, 1560, 1440, 1340, 1240, 1090, 1000, 750 and 720 cm^{-1} ; PMR (CDCl_3) δ 7.1–8.4 (m, 4H) and 6.00 (s, 2H) ppm.

Analysis for $\text{C}_8\text{H}_6\text{BrClO}_2$

Calcd C 38.51 H 2.42,
Found C 38.67 H 2.57.

Chloromethyl m-nitrobenzoate (**1l**): m.p. 42–44 °C, b.p. 154–156 °C (0.8 mm); IR (neat) 3040, 1740, 1610, 1420, 1350, 1250, 1110, 1000, 775 and 725 cm^{-1} ; PMR (CDCl_3) δ 7.6–8.6 (m, 4H) and 6.03 (s, 2H) ppm.

Analysis for $\text{C}_8\text{H}_6\text{ClNO}_4$

Calcd C 44.56 H 2.81 N 6.50,
Found C 44.96 H 3.18 N 6.05.

Chloromethyl p-chlorobenzoate (**1m**): b.p. 88–90 °C (0.3 mm), IR (neat) 2990, 1725, 1580, 1250, 1080, 1010, 845, 755 and 710 cm^{-1} ; PMR (CDCl_3) δ 7.2 to 8.0 (AA'BB', 4H) and 5.93 (s, 2H) ppm.

Analysis for $\text{C}_8\text{H}_6\text{Cl}_2\text{O}_2$

Calcd C 46.86 H 2.95,
Found C 47.11 H 3.18.

Chloromethyl p-bromobenzoate (**1n**): b.p. 108 to 110 °C (0.3 mm), IR (neat) 2990, 1730, 1580, 1395, 1255, 1085, 1010, 850, 760 and 720 cm^{-1} ; PMR (CDCl_3) δ 7.3–8.0 (AA'BB', 4H) and 5.93 (s, 2H) ppm.

Analysis for $\text{C}_8\text{H}_6\text{BrClO}_2$

Calcd C 38.51 H 2.42,
Found C 38.75 H 2.56.

Chloromethyl p-nitrobenzoate (**1o**): m.p. 83–87 °C, chromatographed on magnesium silicate, carbon tetrachloride; IR (KBr) 3060, 1730, 1520, 1340, 1260, 1090, 1010, 990, 860, 735 and 720 cm^{-1} ; PMR (CDCl_3) δ 8.3 (s, 4H) and 6.00 (s, 2H) ppm.

Analysis for $\text{C}_8\text{H}_6\text{ClNO}_4$

Calcd C 44.56 H 2.81 N 6.50,
Found C 44.85 H 3.04 N 6.87.

The high resolution mass measurements of the molecular ions (M^+) for the substituted chloromethyl benzoates are given in Table IV.

Table IV. High resolution mass measurements of the molecular ion (M^+) for substituted chloromethyl benzoates.

Nr.	X =	calcd for	observed	
1g	H	$\text{C}_8\text{H}_7\text{ClO}_2$	170.01341	170.01290
1i	<i>p</i> - CH_3O	$\text{C}_9\text{H}_9\text{ClO}_3$	200.02396	200.01137
1j	<i>p</i> - CH_3	$\text{C}_9\text{H}_9\text{ClO}_2$	184.02905	184.03119
1k	<i>m</i> -Br	$\text{C}_8\text{H}_6\text{BrClO}_2$	247.92392	247.92542
1l	<i>m</i> - NO_2	$\text{C}_8\text{H}_6\text{ClNO}_4$	214.99848	215.00008
1m	<i>p</i> -Cl	$\text{C}_8\text{H}_6\text{Cl}_2\text{O}_2$	203.97444	203.98231
1n	<i>p</i> -Br	$\text{C}_8\text{H}_6\text{BrClO}_2$	247.92392	247.92093
1o	<i>p</i> - NO_2	$\text{C}_8\text{H}_6\text{ClNO}_4$	214.99848	214.99843

2. Determination of the M 301 values

To different quantities of alkylating agents **1a–1h** in dry pear-shaped flasks were added 300 mg of 4-(4-nitrobenzyl) pyridine. The flask was connected

to a condenser and the mixture heated at $70 \pm 0.10^\circ\text{C}$ for 1 h, using a preheated oil bath, then cooled for one minute in an ice-water bath. Acetone (16 ml) was added through the condenser, followed by 1 ml of 50 percent triethylamine in acetone. The purple color which appears immediately was read within two minutes, at 565 nm. All concentrations were measured against distilled water. The standard curves for each agent were constructed using 4–5 different concentrations. The M301 values were obtained from the standard curves.

3. Determination of relative alkylating reactivities (RAR)

To an acetonitrile solution (9 ml) containing 158.8 mg (1.26 mmol) benzyl chloride (6) and 189.0 mg (1.26 mmol) chloromethyl pivalate (1a) was added 212.9 mg (1.26 mmol) 3-acetoxyquinuclidine (5) dissolved in 6 ml acetonitrile. The solution was heated at $70 \pm 0.1^\circ\text{C}$ for 1 h. The acetonitrile was removed under reduced pressure and the residue obtained was dried *in vacuo* over anhydrous calcium sulfate. The residue was dissolved in $\text{D}_2\text{O} \cdot \text{d}^6$ -acetone (1:2 v/v). The composition of the isolated product mixture was determined by multiple integration (5 determinations) of an appropriate resonance signal of the products at expanded sweep widths (250 H_3). Examples are given in Fig. 3.

Fig. 3. The PMR spectrum of the reaction mixture of 1h:1a:3 = 1:1:1. The methylene peaks: a – 5; b – 4h; c – 1a and d – 1h.

Using the procedure described for determining the selectivity of benzyl chloride (6) relative to chloromethyl pivalate (1a) in reaction with 3-acetoxyquinuclidine (5), the haloalkyl carboxylates 1g–1o were also investigated. The corresponding soft quaternary salts were all isolated and identified, as follows:

1-p-Methoxybenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4i): m.p. 185–187 $^\circ\text{C}$ (dec); IR (KBr) 3020, 2990, 1725, 1705, 1590, 1505, 1250, 1170, 1105, 1020 and 770 cm^{-1} ; PMR (D_2O) δ 7.0 to 8.2 (AA'BB', 4H), 5.5 (s, 2H), 3.97 (s, 3H), 3.4–4.4 (6H), 2.3 (s, 3H) and 2.0–3.0 (6H) ppm.

Analysis for $\text{C}_{18}\text{H}_{24}\text{ClNO}_5$

Calcd	C 58.45	H 6.54	N 3.79,
Found	C 58.00	H 6.37	N 3.55.

p-Methylbenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4j): m.p. 201–204 $^\circ\text{C}$ (dec); IR (KBr) 3000, 2980, 1720, 1610, 1370, 1280, 1250, 1120 and 760 cm^{-1} ; PMR (D_2O) δ 7.2–8.2 (AA'BB', 4H), 5.50 (s, 2H), 3.4–4.2 (6H), 2.4 (s, 3H), 2.0–2.8 (6H) and 2.2 (s, 3H) ppm.

Analysis for $\text{C}_{18}\text{H}_{24}\text{ClNO}_4$

Calcd	C 61.10	H 6.84	N 3.96,
Found	C 61.19	H 6.72	N 3.68.

m-Bromobenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4k): m.p. 207–209 $^\circ\text{C}$ (dec); IR (KBr) 2990, 2920, 1725, 1715, 1560, 1280, 1250, 1230, 1085, 1020 and 745 cm^{-1} ; PMR (D_2O) δ 7.4–8.4 (4H), 5.57 (s, 2H), 3.5–4.4 (6H), 2.0–3.0 (6H) and 2.2 (s, 3H) ppm.

Analysis for $\text{C}_{17}\text{H}_{21}\text{BrClNO}_4$

Calcd	C 48.76	H 5.06	N 3.35,
Found	C 48.58	H 5.03	N 3.25.

m-Nitrobenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4l): m.p. 176–179 $^\circ\text{C}$ (dec); IR (KBr) 3040, 2980, 1730, 1605, 1420, 1345, 1260, 1230, 1120, 1040 and 720 cm^{-1} ; PMR (D_2O) δ 7.6–8.8 (4H), 5.63 (s, 2H), 3.4–4.0 (6H), 1.8–3.1 (6H) and 2.2 (s, 3H) ppm.

Analysis for $\text{C}_{17}\text{H}_{21}\text{ClN}_2\text{O}_6$

Calcd	C 53.06	H 5.50	N 7.28,
Found	C 53.40	H 5.72	N 7.01.

p-Chlorobenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4m): m.p. 193–196 $^\circ\text{C}$ (dec); IR (KBr) 3040, 2990, 1725, 1590, 1490, 1400, 1370, 1250, 1100, 1015, 855, 760 and 690 cm^{-1} ; PMR (D_2O) δ 7.4–8.3 (AA'BB', 4H), 5.57 (s, 2H), 3.4–4.2 (6H), 2.0–3.0 (6H) and 2.2 (s, 3H) ppm.

Analysis for $\text{C}_{17}\text{H}_{21}\text{Cl}_2\text{NO}_4$

Calcd	C 54.55	H 5.66	N 3.74,
Found	C 54.50	H 5.51	N 3.54.

p-Bromobenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4n): m.p. 198–201 $^\circ\text{C}$ (dec); IR (KBr) 2980, 1720, 1575, 1255, 1230, 1100, 1005, 840 and 750 cm^{-1} ; PMR (D_2O) δ 7.6–8.2 (AA'BB', 4H), 5.57 (s, 2H), 3.4–4.2 (6H), 2.0–3.0 (6H), and 2.2 (s, 3H) ppm.

Analysis for C₁₇H₂₁BrClNO₄

Calcd C 48.76 H 5.06 N 3.35,
Found C 48.36 H 5.05 N 3.24.

p-Nitrobenzoyloxymethyl-3-acetoxyquinuclidinium
chloride (**4o**): m.p. 179–182 °C (dec); IR (KBr)
2990, 1720, 1510, 1340, 1260, 1230, 1100, 1020,

840 and 710 cm⁻¹; PMR (D₂O) δ 8.4 (s, 4H), 5.70
(s, 2H), 3.4–4.2 (6H), 2.0–3.0 (6H) and 2.3 (s, 3H)
ppm.

Analysis for C₁₇H₂₁ClN₂O₆

Calcd C 53.06 H 5.50 N 7.28,
Found C 53.19 H 5.72 N 7.48.

-
- [1] N. Bodor and J. J. Kaminski, *J. Med. Chem.* **23**, 566 (1980).
[2] N. Bodor, *J. Med. Chem.* **23**, 469 (1980).
[3] J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.* **27**, 1435 (1955).
[4] E. Sawicki, D. F. Bender, T. R. Hauser, R. M. Wilson (Jr.), and J. E. Meeker, *Anal. Chem.* **35**, 1479 (1963).
[5] O. M. Friedman and E. Boger, *Anal. Chem.* **33**, 906 (1961).
[6] R. W. Taft, *J. Phys. Chem.* **64**, 1805 (1960).
[7] K. J. Laidler, *Chemical Kinetics*, McGraw Hill Co., New York 1965.
[8] L. H. Ulich and R. Adams, *J. Am. Chem. Soc.* **43**, 660 (1921).