Quantitative Evaluation of the Reactivity of Alkylating Agents

Nicholas Bodor*, James J. Kaminski, S. D. Worley, and Steven H. Gerson

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

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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 R-COOCH-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:

\[
R_1 \quad R \text{- COOCH-Y + H}_2\text{O} \\
R \text{- COOH + R}_1\text{CHO + HY}
\]

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 alkylation 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):

\[
\text{NBP} \quad \text{O}_2\text{N}\text{-}\text{CH}_2\text{-}\text{CH}_2\text{-}\text{CN} \quad \text{A} \quad \text{X} \quad \text{1/2} \quad \text{B} \quad \text{O}_2\text{N}\text{-}\text{CH} = \text{C} \quad \text{N} \quad \text{-} \quad \text{A}
\]

An alternate method [4] used acetophenone as a solvent and 180°C 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 alkylation 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 alkylation 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...
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 activities of the structurally closely related series of substituted benzoylmethyl halides:

The reaction mixtures of 4g-4o vs 5 were analyzed by NMR, comparing the sharp singlet resonance signals corresponding to the \( N^+ \cdot CH_2 \cdot O \cdot C \cdot R \) methylene, at expanded sweep width. The \(^1\)H and \(^{13}\)C chemical shifts of the methylene group in 1a-1o and the \(^1\)H shifts of the methylene protons in the corresponding soft quaternary salts 4 are shown in Table II.

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

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Chemical shift in 1</th>
<th>Chemical shift in 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>5.70</td>
<td>5.23 (5^c)</td>
</tr>
<tr>
<td>g</td>
<td>5.97</td>
<td>5.47</td>
</tr>
<tr>
<td>i</td>
<td>5.90</td>
<td>5.50</td>
</tr>
<tr>
<td>j</td>
<td>5.90</td>
<td>5.50</td>
</tr>
<tr>
<td>k</td>
<td>6.00</td>
<td>5.57</td>
</tr>
<tr>
<td>l</td>
<td>6.03</td>
<td>5.63</td>
</tr>
<tr>
<td>m</td>
<td>5.93</td>
<td>5.57</td>
</tr>
<tr>
<td>n</td>
<td>5.93</td>
<td>5.57</td>
</tr>
<tr>
<td>o</td>
<td>6.00</td>
<td>5.70</td>
</tr>
</tbody>
</table>

\(^a\) Chemical shifts measured in CDCl\(_3\) solutions.

\(^b\) Chemical shifts measured in D\(_4\)O-D\(_6\)-acetone.

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

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

The \(^1\)H 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 \( \pm 0.5 \) 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 (\( \sigma^2 \))\(^6\) a good correlation was obtained, as shown in Fig. 1.

Benzyl chloride (6) and the unhindered soft alkylation 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>X =</th>
<th>Y</th>
<th>Product composition [%]</th>
<th>RAR (ratio)</th>
<th>PX/PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1g</td>
<td>H</td>
<td>Cl</td>
<td>43 57</td>
<td>0.75 1.00</td>
<td></td>
</tr>
<tr>
<td>1h</td>
<td>H</td>
<td>Br</td>
<td>93 7</td>
<td>13.29</td>
<td></td>
</tr>
<tr>
<td>1i</td>
<td>p-CH₃O</td>
<td>Cl</td>
<td>51 49</td>
<td>1.04 1.28</td>
<td></td>
</tr>
<tr>
<td>1j</td>
<td>p-CH₃</td>
<td>Cl</td>
<td>49 51</td>
<td>0.96 1.28</td>
<td></td>
</tr>
<tr>
<td>1k</td>
<td>m-Bu</td>
<td>Cl</td>
<td>38 62</td>
<td>0.61 0.81</td>
<td></td>
</tr>
<tr>
<td>1l</td>
<td>m-N₂O₂</td>
<td>Cl</td>
<td>31 69</td>
<td>0.45 0.60</td>
<td></td>
</tr>
<tr>
<td>1m</td>
<td>o-Cl</td>
<td>Cl</td>
<td>36 63</td>
<td>0.61 0.75</td>
<td></td>
</tr>
<tr>
<td>1n</td>
<td>p-Br</td>
<td>Cl</td>
<td>38 61</td>
<td>0.61 0.81</td>
<td></td>
</tr>
<tr>
<td>1o</td>
<td>p-N₂O₂</td>
<td>Cl</td>
<td>35 65</td>
<td>0.61 0.72</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C₂H₅CH₂Cl</td>
<td></td>
<td>79 21</td>
<td>3.76</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CH₃(CH₂)₄COOCH₂Cl</td>
<td></td>
<td>35 65</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Plot of relative reactivities (pX/pH) of substituted chloromethyl benzoates vs σ°. Slope = 0.32; r = 0.90.

The relative differences in the ¹³C chemical shifts (Δδ¹³C = δ¹³CX - δ¹³CH) in the series 1h-1o were also plotted against σ°, obtaining a fairly good correlation.

In conclusion, the competitive alkylation provides a sensitive and reliable method for predicting quantitatively the alkylation reactivity of a wide variety of agents. By choosing proper standards, a full scale of reactivity of the known agents can be developed, since the method is certainly not limited to the soft alkylation agents studied. Conventional alkylation 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 k₁/k₂ [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 benzyl chloride and paraformaldehyde using the method described by Ulich and Adams [8], b.p. 150 °C (8 mm)

**Chloromethyl p-methoxybenzoate** (1i): m.p. 110-115 °C (8 mm), IR (KBr) 3030, 2990, 1730, 1580, 1350, 1255, 1085, 1010, 860, 760 and 720 cm⁻¹; PMR (CDCl₃) δ 8.3 (s, 4H) and 6.00 (s, 2H) ppm. Anal. C 44.85 H 3.04 N 6.87.

**Chloromethyl p-bromobenzoate** (1k): m.p. 83-87 °C, chromatographed on magnesium silicate, carbon tetrachloride; IR (KBr) 3060, 1730, 1520, 1340, 1260, 1090, 1010, 990, 860, 755 and 720 cm⁻¹; PMR (CDCl₃) δ 8.3 (4H) and 6.00 (2H) ppm.

**Chloromethyl p-nitrobenzoate** (1o): m.p. 90-91 °C, b.p. 94-95 °C (0.3 mm), IR (KBr) 3060, 1730, 1595, 1340, 1260, 1090, 1010, 990, 860, 755 and 720 cm⁻¹; PMR (CDCl₃) δ 8.3 (4H) and 6.00 (2H) ppm.

**Chloromethyl m-nitrobenzoate** (1l): b.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 (CDCl₃) δ 7.6-8.6 (m, 4H) and 6.03 (s, 2H) ppm.

**Analysis for C₈H₈ClO₄**

Caled C 44.56 H 2.81 N 6.50,

Found C 44.96 H 3.18 N 6.65.

**Chloromethyl p-bromobenzoate** (1m): b.p. 88-90 °C (0.3 mm), IR (neat) 2990, 1725, 1580, 1250, 1080, 845, 755 and 710 cm⁻¹; PMR (CDCl₃) δ 7.2 8.0 (AA’BB’, 4H) and 5.93 (s, 2H) ppm.

**Analysis for C₈H₈ClO₄**

Caled C 46.86 H 2.95,

Found C 47.11 H 3.18.

**Chloromethyl p-nitrobenzoate** (1n): b.p. 110-115 °C (0.8 mm), IR (neat) 2990, 1730, 1595, 1340, 1255, 1085, 1010, 860, 760 and 720 cm⁻¹; PMR (CDCl₃) δ 7.3-8.0 (AA’BB’, 4H) and 5.93 (s, 2H) ppm.

**Analysis for C₈H₈ClO₄**

Caled C 38.51 H 2.42,

Found C 38.75 H 2.57.

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

<table>
<thead>
<tr>
<th>Nr.</th>
<th>X = calcd for</th>
<th>observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1g</td>
<td>H</td>
<td>C₈H₈ClO₂</td>
</tr>
<tr>
<td>1i</td>
<td>p-CH₃O</td>
<td>C₈H₈ClO₂</td>
</tr>
<tr>
<td>1j</td>
<td>p-CH₃</td>
<td>C₈H₈ClO₂</td>
</tr>
<tr>
<td>1k</td>
<td>m-Br</td>
<td>C₈H₈BrClO₂</td>
</tr>
<tr>
<td>1l</td>
<td>m-NO₂</td>
<td>C₈H₈CINO₄</td>
</tr>
<tr>
<td>1m</td>
<td>p-Cl</td>
<td>C₈H₈ClO₂</td>
</tr>
<tr>
<td>1n</td>
<td>p-Br</td>
<td>C₈H₈BrClO₂</td>
</tr>
<tr>
<td>1o</td>
<td>p-NO₂</td>
<td>C₈H₈CINO₄</td>
</tr>
</tbody>
</table>

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 $70 \pm 0.1^\circ$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 (la) was added 212.9 mg (1.26 mmol) 3-acetoxyquinuclidine (5) dissolved in 6 ml acetonitrile. The solution was heated at $70 \pm 0.1^\circ$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 D$_2$O - 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 (la) in reaction with 3-acetoxyquinuclidine (5), the haloalkyl carboxylates 1g–10 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 °C (dec); IR (KBr) 3020, 2990, 1725, 1705, 1590, 1505, 1250, 1170, 1105, 1050 and 770 cm$^{-1}$; PMR (D$_2$O) δ 7.2–8.2 (AA'BB', 4 H), 5.5 (s, 2 H), 3.97 (s, 3 H), 3.4–4.4 (6 H), 2.3 (s, 3 H) and 2.0–3.0 (6 H) ppm.

Analysis for C$_{16}$H$_{24}$ClNO$_5$
Caled 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 °C (dec); IR (KBr) 3000, 2980, 1720, 1610, 1370, 1280, 1250, 1120 and 760 cm$^{-1}$; PMR (D$_2$O) δ 7.2–8.2 (AA'BB', 4 H), 5.5 (s, 2 H), 3.4–4.2 (6 H), 2.4 (s, 3 H), 2.0–2.8 (6 H) and 2.2 (s, 3 H) ppm.

Analysis for C$_{16}$H$_{24}$ClNO$_4$
Caled 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 °C (dec); IR (KBr) 2990, 2920, 1725, 1715, 1500, 1280, 1230, 1085, 1020 and 745 cm$^{-1}$; PMR (D$_2$O) δ 7.4–8.4 (4 H), 5.57 (s, 2 H), 3.5–4.4 (6 H), 2.0–3.0 (6 H) and 2.2 (s, 3 H) ppm.

Analysis for C$_{17}$H$_{21}$BrClNO$_2$
Caled C 48.76 H 5.06 N 3.35,
Found C 48.58 H 5.03 N 3.25.

m-Nitrobenzoyloxy methyl-3-acetoxyquinuclidinium chloride (4l): m.p. 176–179 °C (dec); IR (KBr) 3040, 2980, 1730, 1605, 1420, 1345, 1260, 1230, 1120, 1040 and 720 cm$^{-1}$; PMR (D$_2$O) δ 7.6–8.8 (4 H), 5.63 (s, 2 H), 3.4–4.0 (6 H), 1.8–3.1 (6 H) and 2.2 (s, 3 H) ppm.

Analysis for C$_{17}$H$_{21}$ClNO$_2$
Caled 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 °C (dec); IR (KBr) 3040, 2980, 1720, 1500, 1490, 1400, 1370, 1250, 1100, 1015, 835, 760 and 690 cm$^{-1}$; PMR (D$_2$O) δ 7.4–8.3 (AA'BB', 4 H), 5.57 (s, 2 H), 3.4–4.2 (6 H), 2.0–3.0 (6 H) and 2.2 (s, 3 H) ppm.

Analysis for C$_{17}$H$_{21}$ClNO$_4$
Caled C 54.56 H 5.50 N 7.48,
Found C 54.50 H 5.51 N 7.01.

p-Bromobenzoyloxymethyl-3-acetoxyquinuclidinium chloride (4n): m.p. 198–201 °C (dec); IR (KBr) 3040, 2980, 1725, 1505, 1490, 1400, 1370, 1250, 1100, 1005, 840 and 750 cm$^{-1}$; PMR (D$_2$O) δ 7.6–8.2 (AA'BB', 4 H), 5.57 (s, 2 H), 3.4–4.2 (6 H), 2.0–3.0 (6 H) and 2.2 (s, 3 H) ppm.

Analysis for C$_{17}$H$_{21}$ClNO$_4$
Analysis for C$_{17}$H$_{21}$BrClNO$_4$
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 (D$_2$O) $\delta$ 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 C$_{17}$H$_{21}$ClN$_2$O$_6$
Calcd  C 53.06  H 5.50  N 7.28,
Found  C 53.19  H 5.72  N 7.48.