Original Article

Relationships between Insecticidal Activity of 6-Alkylthio-2-pyridyl Methanesulfonates and Acetylcholinesterase Inhibition of their Sulfone Derivatives against *Nephotettix cincticeps*

Shoichi KATO, Masuko KOSAYASHI, Akio MASUI and Shuichi ISHIDA

Ageo Research Laboratory, Agrochemicals Division, Fine Chemicals Group, Nippon Kayaku Co., Ltd., Koshikiya, Ageo 362, Japan

(Received July 7, 1989)

INTRODUCTION

We have observed previously that 6-alkylthio-2-pyridyl methanesulfonates, their sulfoxides and sulfones are insecticidal, and that some of the sulfonyl analogs also have potent inhibitory activity against acetylcholinesterase prepared from the Ageo (susceptible) and Izumi (resistant) strains of *Nephotettix cincticeps*, whereas the corresponding alkylthio compounds do not. In our quantitative analyses of structure-insecticidal activity relationships of 6-alkylthio compounds in both strains using various physicochemical parameters, the activity was governed similarly in the two strains by the hydrophobicity of the molecule, although it varied parabolically with the optimum alkylthio substituents at C3-C4 alkyl groups. There was a difference in the steric effect of 6-substituents between the two strains: the steric effect of the alkyl group in 6-alkylthio substituents on the activity against the Izumi (resistant) strain was more complex than that against the Ageo (susceptible) strain. In the activity against the Izumi strain, there seemed to be an optimum range in size for the alkyl group, beyond which the activity decreases more sharply than against the Ageo strain.

Recently we prepared fifteen 6-alkylsulfonyl-2-pyridyl methanesulfonates and measured their inhibitory activity (I50) against acetylcholinesterase preparations from the above-mentioned two strains of *N. cincticeps*, aiming to analyze the activity related to physicochemical structural parameters as well as the insecticidal activity of corresponding 6-alkylthio-2-pyridyl methanesulfonates. We also examined correlations between the species. Our finding: the insecticidal mechanism of sulfides is based on the acetylcholinesterase...
inhibition of corresponding sulfones irrespective of strain.

MATERIALS AND METHODS

1. Synthesis of Compounds
Sulfones shown in Table 2 were prepared by the oxidation of the corresponding sulfides, 6-alkylthio-2-pyridyl methanesulfonates, as previously reported.3

2. Biological Tests
The insecticidal activity of 6-alkylthio compounds to the two strains of *Nephotettix cincticeps*, determined previously in terms of log 1/LD$_{50}$ (mol/g insect) by topical application, is relisted in Table 1. The antiacetylcholinesterase activity of the corresponding sulfonyl compounds was measured by the method previously reported.3 The log 1/I$_{50}$ (m) against preparations from homogenates of the Ageo and Izumi strains of *N. cincticeps* is listed in Table 2.

3. Hydrophobicity of Compounds
The log value of capacity factor $k'$ evaluated from retention time by reversed-phase HPLC was used as a hydrophobicity index in quantitative structure-activity relationship analysis. The log $k'$ value of 6-alkylthio compounds was measured previously at the H$_2$O/MeOH (3/7, v/v) of mobile phase. For the value of 6-alkylsulfonyl analogs, the mobile phase was H$_2$O/MeOH (1/1, v/v). All the other conditions were the same as previously reported. Thus, their log $k'$ value was designated as log $k_{802}'$.

RESULTS

1. Quantitative Structure-Activity Relationships in Antiacetylcholinesterase Activity of 6-Alkylsulfonyl-2-pyridyl Methanesulfonates
The antiacetylcholinesterase activity of 6-R-SO$_2$-substituted 2-pyridyl methanesulfonates to enzyme preparations from the Ageo and Izumi strains of *N. cincticeps* varied with the alkyl (R) substituent, as shown in Table 2. The quantitative relationships between the activity index in terms of log (1/I$_{50}$) and physicochemical parameters of these methanesulfonates were examined. Since analogs having singly branched alkyl substituents as R seemed to be more potent than their corresponding isomers, we estimated that there should be optimum steric characteristics. Analyses with various steric parameters along with the hydrophobicity parameter expressed by log $k_{802}'$ showed that the steric constant corrected by Hancock ($E^0_s$) was the best to explain the activity variations, with which Eqs. (1) and (2) were derived. The Hancock steric constant ($E^0_s$) was originally defined to correct possible hyper-conjugation effect of the $\alpha$-hydrogen atom involved in the Taft $E^0$ but it was lately found not to be related to the correction of hyperconjugation but to represent the steric bulk as well as the effect of $\alpha$-branching of substituents.6

Acetylcholinesterase Inhibition of 6-R-SO$_2$-Substituted 2-Pyridyl Methanesulfonates against the Ageo Strain

$$
\log{(1/I_{50})} = -1.660 (E_s^0)^2 - 4.470 E_s^0 \\
-0.887 \log k_{802}' + 6.445
$$

(1)

$n=15$, $s=0.292$, $r=0.934$, $F_{3,11}=24.97$

Acetylcholinesterase Inhibition of 6-R-SO$_2$-Substituted 2-Pyridyl Methanesulfonates against the Izumi Strain

$$
\log{(1/I_{50})} = -1.531 (E_s^0)^2 - 4.406 E_s^0 \\
+5.794
$$

(2)

$n=15$, $s=0.354$, $r=0.933$, $F_{3,12}=40.20$

In these and the following equations, $n$ is the number of compounds, $s$ the standard deviation, $r$ the multiple correlation coefficient, and $F_{v1,v2}$ the F value of correlation where $v_1=m$ and $v_2=n-m-1$: $m$ is the number of independent variables used in correlation. The figures in parentheses are 95% confidence intervals of the corresponding constant.

From Eqs. (1) and (2), optimum $E^0_s$ values were estimated to be almost the same: $-1.35$ for the Ageo strain and $-1.44$ for the Izumi strain. The negative coefficient of the log $k_{802}'$ term in Eq. (1) means that the activity to the susceptible strain decreases with an increase in hydrophobicity of the molecule.
Table 1  Insecticidal activity of 6-R-S-substituted 2-pyridyl methanesulfonates against *Nephotettix cincticeps*.

![Structural parameters](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Substituent</th>
<th>Structural parameters</th>
<th>log 1/LD&lt;sub&gt;50&lt;/sub&gt; (M)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ageo</th>
<th>Izumi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E&lt;sub&gt;S&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>log k&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Obsd.</td>
</tr>
<tr>
<td>1</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.00</td>
<td>-0.259</td>
<td>6.71</td>
<td>6.85</td>
</tr>
<tr>
<td>2</td>
<td>C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>-0.38</td>
<td>-0.034</td>
<td>8.14</td>
<td>7.88</td>
</tr>
<tr>
<td>3</td>
<td>n-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;</td>
<td>-0.67</td>
<td>0.196</td>
<td>8.57</td>
<td>8.36</td>
</tr>
<tr>
<td>4</td>
<td>i-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;</td>
<td>-1.08</td>
<td>0.163</td>
<td>8.43</td>
<td>8.55</td>
</tr>
<tr>
<td>5</td>
<td>n-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;</td>
<td>-0.70</td>
<td>0.425</td>
<td>8.30</td>
<td>8.19</td>
</tr>
<tr>
<td>6</td>
<td>s-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;</td>
<td>-1.74</td>
<td>0.370</td>
<td>8.24</td>
<td>8.26</td>
</tr>
<tr>
<td>7</td>
<td>i-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;</td>
<td>-1.24</td>
<td>0.399</td>
<td>8.16</td>
<td>8.32</td>
</tr>
<tr>
<td>8</td>
<td>t-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;</td>
<td>-2.46</td>
<td>0.277</td>
<td>7.82</td>
<td>8.00</td>
</tr>
<tr>
<td>9</td>
<td>n-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;</td>
<td>-0.71</td>
<td>0.649</td>
<td>7.43</td>
<td>7.33</td>
</tr>
<tr>
<td>10</td>
<td>i-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;</td>
<td>-0.66</td>
<td>0.614</td>
<td>7.24</td>
<td>7.38</td>
</tr>
<tr>
<td>11</td>
<td>n-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;)CH</td>
<td>-1.63</td>
<td>0.590</td>
<td>7.65</td>
<td>7.84</td>
</tr>
<tr>
<td>12</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-0.69</td>
<td>0.330</td>
<td>7.91</td>
<td>8.34</td>
</tr>
<tr>
<td>13</td>
<td>c-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;</td>
<td>-1.12</td>
<td>0.447</td>
<td>8.39</td>
<td>8.23</td>
</tr>
<tr>
<td>14</td>
<td>c-C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-1.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.192</td>
<td>8.64</td>
<td>8.58</td>
</tr>
<tr>
<td>15</td>
<td>c-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;</td>
<td>-1.40</td>
<td>0.664</td>
<td>7.69</td>
<td>7.48</td>
</tr>
<tr>
<td>16</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>-0.69</td>
<td>0.268</td>
<td>8.53</td>
<td>8.22</td>
</tr>
<tr>
<td>17</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-0.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.174</td>
<td>7.70</td>
<td>7.76</td>
</tr>
<tr>
<td>18</td>
<td>t-C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-2.05</td>
<td>0.558</td>
<td>8.26</td>
<td>7.77</td>
</tr>
</tbody>
</table>

<sup>a</sup> By topical application.  <sup>b</sup> Hancock steric constant taken from Ref. 4 and calculated from E<sub>S</sub> value [Refs. 9 and 10] by the equation, E<sub>S</sub> = E<sub>S</sub> - 0.306(3 - n<sub>H</sub>), where n<sub>H</sub> is the number of α-hydrogen atoms.  <sup>c</sup> Mobile phase: H<sub>2</sub>O/MeOH (3/7, v/v).  <sup>d</sup> By Eq. (3).  <sup>e</sup> By Eq. (5).  <sup>f</sup> By Eq. (4).  <sup>g</sup> By Eq. (6).  <sup>h</sup> Taken as that of i-C<sub>4</sub>H<sub>9</sub>.  <sup>i</sup> Taken as that of n-C<sub>4</sub>H<sub>9</sub>.
2. Quantitative Relationships between Insecticidal and Anticholinesterase Activities

Relationships between the insecticidal activity of 6-R-S-substituted 2-pyridyl methanesulfonates (1-13, 15 and 16) and the anticholinesterase activity of the corresponding sulfone derivatives (19-33) were formulated by Eqs. (3) and (4).

Insecticidal Activity of 6-R-S-Substituted Compounds and Acetylcholinesterase Inhibition of their Sulfone Counterparts to the Ageo Strain

\[
\log(1/LD_{50}) = -0.246 \log(1/I_{50}) - 4.933 \log k'_{50}^a + 5.980 \log k'_{50}^a
\]

\( (0.234) \quad (1.926) \quad (1.215) \quad (1.921) \)

\( n=15, s=0.240, r=0.915, F_{s,11}=18.89 \)

Insecticidal Activity of 6-R-S-Substituted Compounds and Acetylcholinesterase Inhibition of their Sulfone Counterparts to the Izumi Strain

\[
\log(1/LD_{50}) = -0.626 \log(1/I_{50}) - 2.258 \log k'_{50}^a + 2.501 \log k'_{50}^a
\]

\( (0.214) \quad (1.307) \quad (1.658) \)

\( n=15, s=0.295, r=0.880, F_{s,12}=20.58 \)

Eqs. (3) and (4) indicate that the insecticidal activity of sulfide to both strains is highly related to the acetylcholinesterase inhibition of corresponding sulfone when the hydrophobicity factor of the molecule is taken into account. Without addition of \( \log k' \) terms in Eqs. (3) and (4), the correlation was much poorer: \( s=0.454 \) and \( r=0.563 \) for the Ageo strain, and \( s=0.418 \) and \( r=0.713 \) for the Izumi strain, respectively.


As mentioned above, the acetylcholinester-
ase inhibitory activity in terms of log(1/\text{LD}_{50}) of sulfones showed a good correlation when the $E_s^s$ value was used as a steric effect parameter. In our previous analysis\(^2\) of the structure-insecticidal activity correlation of 6-R-S-substituted compounds, the STERIMOL $B_1$ and $B_5$, and the Taft $E_0$ were used to describe the steric effect. Thus, the correlations were reexamined by using $E_s^s$ values without omitting and compounds in Table 1 to formulate Eqs. (5) and (6).

### Insecticidal Activity to the Ageo Strain

$$\log (1/\text{LD}_{50}) = -4.561 (\log k')^2 + 1.746 \log k'$$

(1.948) (1.304)

$$-0.462 (E_s^s)^2 - 1.276 E_s^s$$

(0.368) (1.054)

$$+ 7.539$$

(0.320)

$$n=18, s=0.254, r=0.903, F_{4,13}=14.29$$

### Insecticidal Activity to the Izumi Strain

$$\log (1/\text{LD}_{50}) = -3.778 (\log k')^2 + 1.629 \log k'$$

(2.413) (1.615)

$$-0.712 (E_s^s)^2 - 1.926 E_s^s$$

(0.456) (1.306)

$$+ 6.303$$

(0.644)

$$n=18, s=0.315, r=0.879, F_{4,13}=11.03$$

Collinearity between variables is shown in Table 3. Both Eqs. (5) and (6) indicate that variations in the activity are parabolically related to the hydrophobicity as well as the steric bulk including the $\alpha$-branching factor of R. The optimum values of $\log k'$ and $E_s^s$ in Eq. (5), 0.19 and $-1.38$, are close to the corresponding values in Eq. (6), 0.22 and $-1.35$ respectively, being nearly equal in the Ageo and Izumi strains. In Eq. (5), the use of $B_1$ or $E_s$ instead of $E_s^s$ values gave lower $r$ values, 0.897 or 0.843, but the use of $B_1$ presented almost the same $r$ and $s$ values, 0.904 and 0.253, respectively. In Eq. (6), the use of $B_1$, $B_5$ or $E_s$ in the place of $E_s^s$ yielded poorer correlation, $r=0.854, 0.827$ or 0.862.

### DISCUSSION

From the above comparative correlations, we concluded that the insecticidal activity of the 6-alkylthio compounds is indeed due to the antiacetylcholinesterase activity of the corresponding 6-alkylsulfonyl derivatives.

The steric parameter terms are almost identical between Eqs. (1) and (2), indicating that steric demand expressed by the bulk and the $\alpha$-branching of the alkyl moiety of substituents is very similar in the two strains as far as the antiacetylcholinesterase activity of 6-alkylsulfonyl compounds is concerned. The susceptible enzyme could accommodate the inhibitor sterically in a manner similar to the Izumi (resistant) enzyme. The fact that the negative hydrophobic term was required only for the inhibition against the Ageo (susceptible) strain in Eq. (1), however, shows a difference in the enzyme inhibition process between the strains. The active site of the susceptible enzyme may be more hydrophilic than that of the resistant enzyme. The antiacetylcholinesterase activity of o-, m- and $p$-substituted phenyl N-methylcarbamates against smaller brown planthoppers was related to the hydrophobicity ($\pi$) of substituents, and the coefficient of the $\pi$ terms of $p$-substituents was negative, whereas those of o- and m-substituents was positive.\(^11\) It is suggested that $p$-substituents interact hydrophobically with a region lacking in continuity with regions interacting with o- and m-substituents so as to oppose the interaction of the carbamoyl group with the active serine OH of the enzyme.\(^13\)

Equations (3) and (4) show that the insecticidal activity of the 6-alkylthio compounds is governed by the enzyme inhibition of the corresponding sulfonyl derivatives as well as the transport factor of the applied 6-alkylthio compounds expressed by the quadratic terms(s) of $\log k'$. The partial regression coefficient of log(1/\text{LD}_{50}) term in Eq. (3) for the Ageo strain is 0.246 whereas that in Eq. (4) for the Izumi strain is 0.626. Exclusive of the transport factor, contribution of enzyme inhibition to

<table>
<thead>
<tr>
<th>$\log k'$</th>
<th>$(\log k')^2$</th>
<th>$E_s^s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.716</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>0.097</td>
<td>0.024</td>
<td>1.000</td>
</tr>
<tr>
<td>0.211</td>
<td>0.055</td>
<td>0.918</td>
</tr>
</tbody>
</table>

Table 3 Squared correlation ($r^2$) matrix for variables used to derive Eqs. (5) and (6).
the insecticidal activity was much lower in the susceptible strain than in the resistant strain. The difference between the sensitive and resistant strains was actually reflected in the difference in intercept and transport terms in Eqs. (3) and (4). The intercept in Eq. (3) for the susceptible strain is greater by about 3.5 than that in Eq. (4) for the resistant strain. Furthermore, the higher the hydrophobicity of the molecule from log $k'=0$, the lower the insecticidal activity against the resistant strain in Eq. (4), whereas the insecticidal activity against the susceptible strain is enhanced until it reaches about 0.22 in Eq. (3). These factors in combination seem to reduce the insecticidal activity to the resistant strain. No steric parameter was required in Eqs. (3) and (4), suggesting that no specific steric effect is operative in the metabolic oxidation mechanism of 6-alkylthio substituents leading to the corresponding 6-alkylsulfonyl groups, which is regarded as the molecular species responsible to enzyme inhibition. However, it is also possible that differences in metabolism, including activation from sulfide to sulfone, or detoxication by hydrolysis of sulfone in the two strains are not separated in Eqs. (3) and (4).

Equations (5) and (6) show that the optimum hydrophobic and steric natures of the 6-alkyl moiety of the molecule for insecticidal activity are very close in the susceptible and resistant strains. However, this result is regarded to occur after compensation of factors governing the underlying unit processes. In the final enzyme inhibition, hydrophobicity of the molecule has a negative effect on the susceptible strain. In the transport process, hydrophobicity is to a certain extent more favorable to the susceptible strain than to the resistant strain. These effects on overall insecticidal activity in total do not appear in Eqs. (5) and (6). The most significant difference between Eqs. (5) and (6) is that the coefficient value of the $(E^*)^2$ term of Eq. (6) for the resistant strain is about twice more negative than that of Eq. (5). This means that the overall insecticidal activity decreases more sharply in the resistant strain than in the susceptible strain to the steric effects at the both sides of the optimum values. This difference is not due to the difference in enzyme inhibition but attributable to the difference that arises as the total of overall activity.

In summary, physicochemical factors governing the variations in overall insecticidal activity of this series of compounds are similar in the two strains. In this respect the 6-cyclopentylmethylthio derivative (14), whose $E^*$ and log $k'$ values are close to their respective optimum values, seems to be an optimized compound.

ACKNOWLEDGMENTS

We wish to express our thanks to Professor Toshio Fujita of Kyoto University for his invaluable discussions and reviewing the manuscript. We also thank Mr. Mineo Maruyama, Miss Chiaki Tanaka and other colleagues of our Agrochemicals Division of Nippon Kayaku Co., Ltd. for their support in this work.

REFERENCES

要　約

ツマグロヨコバイに対する 6-アルキルチオ-2-ビリジルメタンスルホナート類の殺虫活性と、それらのスルホン誘導体のアセチルコリンエステラーゼに対する阻害活性との関係*

加藤彰一、小林益子、桝井昭夫、石田秀司

6-アルキルチオ-2-ビリジルメタンスルホナート類のスルホン体15化合物を合成し、上尾系および出水系ツマグロヨコバイのアセチルコリンエステラーゼに対する阻害活性 (I50) を測定した。両系に対する 6-アルキルチオ体の殺虫活性は、相当するスルホン体のアセチルコリンエステラーゼ阻害活性と輸送に関与するであろう分子の疎水性 (log k') により良好に説明できた。さらにいずれの系においても、この 6-アルキルチオ体の殺虫活性は、酵素阻害と輸送過程に関与する疎水性項と立体パラメーター項の組合せで表わすことができた。

* スルホナート殺虫剤の構造活性相関（第4報）