Research Article

Gas Chromatography Electron Ionization Mass Spectral Analysis of Thio Analogues of Pyrimidine Bases: 5-Bromo-2,4-di-o-(m- and p-) chloro- (bromo-)benzylthiouracils and 6-methyluracils

G. Bartkowiak, E. Wyrzykiewicz, and G. Schroeder

Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

Correspondence should be addressed to G. Bartkowiak, gbartkow@amu.edu.pl

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Electron ionization (EI) mass spectral fragmentation routes of twelve 5-bromo-2,4-di-o-(m- and p-) chloro- (bromo-)benzylthiouracils and 6-methyluracils are investigated. The compounds studied are analyzed using gas chromatography/mass spectrometry (GC/MS). Fragmentation pathways, whose elucidation is assisted by accurate mass measurements and metastable transitions, are discussed. Correlation between the abundances of the selected fragment ions of the compounds investigated is discussed. The data obtained make grounds for distinction of structural isomers.

1. Introduction

Thio derivatives of pyrimidine bases are of interest because of their biological and pharmacological activities, for example, as minor components of t-RNA or as antithyroidal and anticancer drugs as well as sedatives [1–7]. Essential role in biological systems play compounds, which contain 5-bromopyrimidine moiety, like 5-bromouracil and 5-bromouridine. It is well known that these compounds are mutagenic [8–10] and able to replace thymine residue in the DNA molecule. On the other hand, C-5 bromo group is a hydrophobic substituent having also electron-negative properties, very important for anaesthetic and anticonflict activities [11]. The presence of benzyl group also influences the activity of pyrimidine derivatives, for example, the pyrimidine thioethers with 2-benzylthio substituent have been reported as a novel nonnucleoside HIV-1 reverse transferase inhibitors (NNRTIs) with activity against BHAP-resistant HIV [12, 13]. Because of biological importance of the modified thio analogues of pyrimidine bases, much attention has been devoted to recognize their properties. Mass spectrometry continues to be a convenient and effective method for determination of nature of covalent modifications to thio analogues of nucleobases and for distinction of structural isomers [14–17]. The mass spectral behavior of modified derivatives of thionucleobases, for example, 2-benzylthio and 4-benzylthiouracils which may appear in the gas phase in various tautomeric forms has been studied previously. However, to the best of our knowledge, no work has been published about the mass spectrometric behavior of fully aromatic 2,4-dibenzythio-5-bromouracils. The hereby presented study of mass fragmentation of the title compounds were undertaken to examine the influence of C-5 bromo substitution in pyrimidine ring on their EI mass fragmentation.

Isomeric organic compounds discrimination is a challenging task for mass spectrometrists because of the identical molecular masses and similar fragmentation pathways of these species. However, differences in the nature and electronic effects of substituents, depending on their location in the molecule, lead to different stabilization possibilities and influence the kinetics of mass dissociation of molecular and fragment ions. These factors are strongly reflected by the abundances of ions in the electron-ionization mass spectra of such molecules. Comparison of the abundances of selected fragment and molecular ions reveals the preferences of the molecule’s breakup and often allows isomers differentiation [18, 19].

The aim of this investigation was to elucidate the EI mass spectrometric fragmentations of 5-bromo-2,4-di-o-(m- and p-) chloro- (bromo-)benzylthiouracils 1–6 and
5-bromo-2,4-di-o-(m- and p-) chloro-(bromo-) benzylthio-
6-methyluracils 7–12 (presented in Figure 1) and to find out
whether it is possible to differentiate isomeric species in this
group of compounds on the basis of their EI mass spectra.
A comparison was made of the values of coefficients μ,
that is, the ratios of the abundances of selected ions, fragment or
molecular, to those of other characteristic ions. This proce-
dure has been previously used in our laboratory [20–23].

To ensure that the EI mass spectra of 1–12 come from the
really pure compounds, all the above 2,4-dithiouracil deriva-
tives obtained have been submitted to gas chromatography
combined with electron ionization mass spectrometry.

2. Experimental

All compounds 1–12 were analyzed on a gas chromatograph
linked with a mass spectrometer Varian 4000 GC/MS. Gas
chromatography conditions were GC CP3800 equipped
with column VF-5 ms (30 m × 0.25 mm × 0.39 mm),
carrier gas helium, 1 mL min⁻¹, injector type 1177, split
1:50, temperature 250°C. Low-resolution mass spectra were
taken from the individual chromatographic peaks after
GC isolation/purification of compounds studied. The mass
spectrometer with internal ion trap worked with electron
ionization at the ion source temperature 220°C and ion-
ization energy 70 eV. The measurements were made in the
mass range 40–800 m/z. High-resolution mass spectra and
metastable transitions were recorded on an AMD-Intectra
GmbH (D-27243 Harpstedt, Germany) Model 402 two-
sector mass spectrometer (ionizing energy 70 eV, accelerating
voltage 8 kV, resolution 10.000 at 10% valley). Samples were
introduced using a direct insertion probe at a source temper-
ature ~160°C. The elemental compositions of the ions were
determined by peak matching relative to perfluorokerosene.
All masses measured agreed with those of the compositions
given in Tables 1 and 2 within ±2 ppm. Data from the first
field free region, recorded using constant B/E and B²/E
linked scans, were obtained.

The values of μ (see further in the text) were calculated as
averages of three measurements (Table 3). Compounds 1–12
were obtained according to literature [24].

3. Results and Discussion

On the basis of the low-resolution EI mass spectra as well
as B/E and B²/E = const linked scan spectra and exact
mass measurements (Tables 1 and 2), the principal EI mass
fragmentation routes of compounds 1–12 are interpreted
as shown in Scheme 1. Bearing in mind that the structures
of fragment ions are always speculative, only elemental
compositions for the fragment ions are given. The electron-
ionization mass fragmentation pathways of M⁺ (a) ions of
1–12 confirmed by the metastable transitions observed in
B/E and B²/E linked scan spectra are labeled with an asterisk
in Scheme 1. The abundances of the selected important ions
are shown in Tables 1 and 2. It should be emphasized that
theoretical isotopic patterns are much more complex than
those shown in Tables 1 and 2, but, for clarity, only the main,
most intense, isotopic peaks have been taken into account.
The isotopic distribution of some ions, for example, b (M⁺–
*SH) and c (M⁺–*Cl) of compounds 1–3 are disturbed due
to their signals overlapping.

As an example, theoretical isotopic pattern [m/z (relative
intensity), the most intense bolded] of ion b
(C₁₈H₁₂N₂SBrCl₂) is

437(59.2%)/438(12.5%)/439(100%)
/440(20.9%)/441(50%)/442(10.2%)/443(9%) (1)
/444(1.7%)/445(0.4%)/446(0.1%)
### Table 1: Elemental compositions and relative abundances of the ion peaks in the spectra of 1–6 according to the high-resolution data.

<table>
<thead>
<tr>
<th>Ion</th>
<th>m/z</th>
<th>Elemental composition</th>
<th>% relative abundance (%RA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>M⁺⁺</td>
<td>470/472/474</td>
<td>C₉₋₁₂H₁₉N₂S₂Cl₂Br</td>
<td>13/20/13</td>
</tr>
<tr>
<td>a</td>
<td>558/560/562/564</td>
<td>C₉₋₁₂H₁₉N₂S₂Br₂</td>
<td>—</td>
</tr>
<tr>
<td>b [a−SH]</td>
<td>437/439/441</td>
<td>C₉₋₁₂H₁₉N₂S₂Cl₂Br</td>
<td>27/14/3</td>
</tr>
<tr>
<td>c [a−X]</td>
<td>435/437/439</td>
<td>C₉₋₁₂H₁₉N₂S₂Br₂</td>
<td>21/27/14</td>
</tr>
<tr>
<td>d [a−SH−X]</td>
<td>402/404/406</td>
<td>C₉₋₁₂H₁₉N₂S₂Cl₂Br</td>
<td>3/4/1</td>
</tr>
<tr>
<td>d' [a−SH−HX]</td>
<td>401/403/405</td>
<td>C₉₋₁₂H₁₉N₂S₂Cl₂Br</td>
<td>2/5/3/4</td>
</tr>
<tr>
<td>e</td>
<td>345/347/349</td>
<td>C₉₋₁₂H₁₉N₂S₂Cl₂Br</td>
<td>18/25/9</td>
</tr>
<tr>
<td>f [e−X]</td>
<td>310/312</td>
<td>C₉₋₁₂H₁₉N₂S₂Br</td>
<td>19/20</td>
</tr>
<tr>
<td>g</td>
<td>125/127</td>
<td>C₇H₆Cl</td>
<td>100/36</td>
</tr>
<tr>
<td>h</td>
<td>90</td>
<td>C₇H₆</td>
<td>58</td>
</tr>
</tbody>
</table>

### Table 2: Elemental compositions and relative abundances of the ion peaks in the spectra of 7–12 according to the high-resolution data.

<table>
<thead>
<tr>
<th>Ion</th>
<th>m/z</th>
<th>Elemental composition</th>
<th>% relative abundance (%RA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>M⁺⁺</td>
<td>484/486/488</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Cl₂Br</td>
<td>20/41/24</td>
</tr>
<tr>
<td>a</td>
<td>572/574/576/578</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Br₂</td>
<td>—</td>
</tr>
<tr>
<td>b [a−SH]</td>
<td>451/453/455</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Cl₂Br</td>
<td>12/9/4</td>
</tr>
<tr>
<td>c [a−X]</td>
<td>449/451/453</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Br₂</td>
<td>3/12/9</td>
</tr>
<tr>
<td>d [a−SH−X]</td>
<td>416/418/420</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Cl₂Br</td>
<td>4/4/3</td>
</tr>
<tr>
<td>e</td>
<td>359/361/363</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Cl₂Br</td>
<td>31/33/14</td>
</tr>
<tr>
<td>f [e−X]</td>
<td>324/326</td>
<td>C₁₉₋₂₁H₁₅N₂S₂Br</td>
<td>18/18</td>
</tr>
<tr>
<td>g</td>
<td>125/127</td>
<td>C₇H₆Cl</td>
<td>100/31</td>
</tr>
<tr>
<td>h</td>
<td>90</td>
<td>C₇H₆</td>
<td>57</td>
</tr>
</tbody>
</table>

### Table 3: Values of coefficients μ (definitions in the text) for 5-bromo-2,4-di-o-(m- and p-) chloro- (bromo)benzylthiouracils 1–12.

<table>
<thead>
<tr>
<th>μ</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ₁ = a/g</td>
<td>0.20</td>
<td>0.42</td>
<td>0.24</td>
<td>0.30</td>
<td>0.47</td>
<td>0.38</td>
<td>0.41</td>
<td>0.48</td>
<td>0.33</td>
<td>0.60</td>
<td>0.67</td>
<td>0.45</td>
</tr>
<tr>
<td>μ₂ = e/g</td>
<td>0.25</td>
<td>0.42</td>
<td>0.24</td>
<td>0.54</td>
<td>0.84</td>
<td>0.48</td>
<td>0.33</td>
<td>0.78</td>
<td>0.46</td>
<td>0.89</td>
<td>1.37</td>
<td>0.73</td>
</tr>
<tr>
<td>μ₃ = f/g</td>
<td>0.20</td>
<td>0.13</td>
<td>0.09</td>
<td>0.68</td>
<td>0.18</td>
<td>0.48</td>
<td>0.18</td>
<td>0.13</td>
<td>0.05</td>
<td>0.77</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>μ₄ = f/a</td>
<td>1.00</td>
<td>0.31</td>
<td>0.38</td>
<td>2.26</td>
<td>0.38</td>
<td>0.16</td>
<td>0.43</td>
<td>0.27</td>
<td>0.15</td>
<td>1.29</td>
<td>0.24</td>
<td>0.27</td>
</tr>
</tbody>
</table>
and, for ion c (C_{18}H_{13}N_{2}S_{2}BrCl)

\[ \text{m/z 435}(70.9\%) / \text{m/z 436}(15.6\%) / \text{m/z 437}(100\%) \]

\[ / \text{m/z 438}(21.6\%) / \text{m/z 439}(32.8\%) / \text{m/z 440}(6.7\%) \]

\[ / \text{m/z 441}(2.8\%) / \text{m/z 442}(0.4\%) / \text{m/z 443}(0.1\%) \]

so the abundances of ions at m/z 437 and 439 add up.

As can be seen from Scheme 1 and the data presented in Tables 1 and 2, the principal mass fragmentation pathways of 1–6 and 7–12 are similar but show differences in the abundances of important fragment ions. The fragmentation of molecular ion of the compounds studied 1–12 proceeds according to the same pattern (as can be seen from Figures 2(a) and 2(b), presenting EI mass spectra of two of the compounds studied as representative examples), in spite of the different substituents (chloro- or bromo-) and different positions (ortho or para) of halogen in the phenyl ring. Figure 2(a) shows the EI mass spectrum of 5-bromo-2,4-di-o-chlorobenzylthio-6-methyluracil (compound 7) and Figure 2(b) the EI mass spectrum of 5-bromo-2,4-di-p-bromobenzylthio-6-methyluracil (compound 12). In the figures mentioned above, the main fragmentation features of M⁺ are clearly seen: both molecular ions easily lose *SH and *X (*Cl or *Br, resp.) radicals as well as *CH_{2}C_{6}H_{4}X, that is, the whole halobenzyl group. Other losses are loss of *SH radical and neutral molecule HX (M⁺⁺–*SH–HCl or M⁺⁺–*SH–HBr) and combined loss of halobenzyl radical and halogen from the other halobenzyl group in the molecule, however, it seems preferred that the loss of *Br occurs rather from bromobenzyl derivatives and the loss of HCl occurs more likely from chlorobenzyl compounds. The same tendency is seen in the second step of fragmentation: the loss of HCl from the ions g m/z 125 (CH_{2}C_{6}H_{4}Cl) gives daughter ion j m/z 89 (C_{7}H_{4}⁺) and loss of *Br from the ions at m/z 169/171 (CH_{2}C_{6}H_{4}Br) yields ion h at m/z 90 (C_{7}H_{4}⁺⁺), which confirms a known fact that HCl formation is thermochemically favoured.

The B/E = const linked scan spectrum of molecular ion of compound 12 (Figure 1, see Supplementary Material available online at doi: 10.1155/2012/847676), with regard to the lowest mass isotope of bromine, that is, /Br₂, presents the first step of fragmentation, that is, daughter ions formed through the dissociation of a (C_{19}H_{15}N_{2}S_{2} /Br₂, m/z 572). It indicates that most of important and abundant fragment ions, listed in Table 2, originate directly from the molecular ion. A comparative set of the EI mass spectra of 4–6 is presented in Figure II(A–C), Supplementary Material. Note different abundances of corresponding fragment ions and different retention times.

One of the essential processes of EI-MS decomposition of molecular ions 1–12 is the elimination of *SH radicals.

Scheme 1: Pathways of the EI mass fragmentation of the molecular ions M⁺⁺ (a) of 1–12.
For the loss of •SH, a skeletal rearrangement is required which implies the formation of new carbon-carbon and carbon-nitrogen bonds. The •SH elimination occurs directly from molecular ions a, leading to the even-electron fragment ions b, as well as from the many fragment ions, giving second generation of daughter ions of low abundance in the EI mass spectrum. Fragment ions b, that is, [M•–•SH], are precursors of ions d, created through the loss of •X from b. According to the metastable transition spectra (B/E linked scan method), the loss of •SH is a widespread process in the fragmentation routes of 1–12 because it takes place also for ions c, d, e, and f, although the generated daughter ions d1, e1, f1 are of low abundance and low significance for differentiation of isomers, and therefore they are not listed in Tables 1 and 2. It should be mentioned that the loss of a sulfhydryl radical is common for aromatic thioethers [25] and is a characteristic feature of EI mass fragmentation of molecular ions of alkylthio-5-bromo and alkoxy carbonylalkylthiouracils [23, 26] as well as 2-benzylthioorotic acids [27].

Molecular ions a decompose also with halogen radical elimination. In the EI mass spectra of compounds 4–6 and 9–12, the evident loss of •Br from molecular ion is seen, especially pronounced for the ortho isomers 4 and 10. The bromine loss can be noticed also in the further steps of fragmentation, for example, the B/E = const linked scan spectrum of ion c of compound 12 presents intense •Br elimination. It remains unknown if there is a loss of bromine radical from the uracil or phenyl ring. Analysis of EI mass spectra of 1–3 and 7–9, where the [M•–Br] ions are almost completely absent, indicates that bromine originates from benzyl moiety. Analogously, ions e of 10–12, that is, C12H9N2S2Br2 lose bromine radical giving f, but ions e of 7–9 lose •Cl not •Br. It means that in this series of uracil derivatives bromine radicals are more easily abstracted from phenyl than from pyrimidine ring and the X-Csp2 bond in phenyl ring is more prone to cleave than in heteroaromatic one. This is the difference between disubstituted 5-bromodithiouracils and monosubstituted 5-bromothiouracils. It was mentioned earlier that •Br loss from C-5 position of pyrimidine ring is characteristic of many bromouracil and bromothiouracil derivatives and that •Br radical elimination is more intense for 4-thio- than for 2-thioderivatives [23, 26]. For molecular ions of 5-bromo-2,4-di-o- (m- and p-) chloride (bromo-) benzylthiouracils, the elimination of bromine radical from position 5 of pyrimidine ring does not occur in the EI-MS.
It is clear that, during the simple cleavage of the C–S bonds (I) and the odd-electron loss of HX molecule to the odd-electron fragment ions (II), positive charge is stabilized more efficiently on the halobenzyl fragment. Ions (III) undergo further fragmentation through the elimination of *X radical or the neutral loss of HX molecule to the odd-electron fragment ions (IV), and even-electron fragment ions (V), respectively. Ions (VI) decompose further losing sulphydryl (*SH), substituted benzyl (*C7H6X), or halogen (*X) radicals. Odd-electron fragment ions (VII) undergo the elimination of *SH, *X (or HX), *SH and *X sequentially as well as they lose neutral fragments C7H5SX. The created daughter ions are of low abundance (and that is why they are not listed in Tables 1 and 2).

The differences in the fragmentations within each of the four sets of isomeric compounds within the series of 5-bromo-2,4-di-o- (m- and p-) chloro- (bromo-) benzthiouracils 1–6 and 5-bromo-2,4-di-o- (m- and p-) chloro- (bromo-) benzthio-6-methyluracils 7–12 were expressed quantitatively by comparing the calculated values of the coefficients μ, that is, ratios of the relative abundances (RA) of chosen ions. Relative abundance RA is defined as % of the abundance of a given ion relative to that of the most abundant ion in the spectrum, whose abundance is assumed as 100%. In this work, we decided to refer relative abundances of important ions: a (molecular ions, M**) and e (M***) (M***) (CH2C6H4X) to the relative abundances of very stable ions g, that is, halobenzyl cations (or, equivalently, halotropylium ions). We have also observed that ions f are the most abundant for all ortho isomers of compounds 1–12, that is, for compounds 1, 4, 7, and 10 (Tables 1 and 2), and that is why we defined two coefficients based on the f ions: f/g and f/a.

The μ coefficients, used for isomers studied discrimination, are defined as follows:

\[
\begin{align*}
\mu_1 &= \frac{\%RA_{a}}{\%RA_{g}}, \\
\mu_2 &= \frac{\%RA_{e}}{\%RA_{g}}, \\
\mu_3 &= \frac{\%RA_{f}}{\%RA_{g}}, \\
\mu_4 &= \frac{\%RA_{f}}{\%RA_{a}}.
\end{align*}
\]

In each set of three isomers (1–3, 4–6, 7–9, 10–12) meta isomer can be distinguished from ortho and para by the highest value of μ1 and μ2. For all dithiouracils derivatives studied 1–6, the values of μ1 are decreasing in the order meta > para > ortho, and, for the substituted dithio-6-methyluracils 7–12, descending order of μ1 is meta > ortho > para.

The coefficient μ2 is evidently the highest for all meta isomers and comparable for ortho and para. So one can describe the order of μ2 values as:

\[
\mu_2 \text{ meta} \gg \mu_2 \text{ ortho} \approx \mu_2 \text{ para} \quad 1–12.
\]

The conclusion is that μ1 and μ2 are sufficient for distinction isomers meta from ortho and para but insufficient for discrimination between para and ortho. However, the ortho isomers can be distinguished from the others by the highest value of μ3 and μ4.

It should be emphasized that compounds 1–12 are thermally stable under the mass spectrometric conditions.
4. Conclusions

The present study has demonstrated that the analysis of EI-induced mass spectra of 5-bromo-2,4-di-o - (m - and p -) chloro- (bromo-) benzylthiouracils (1–6) and 6-methyluracils (7–12) is a useful method for distinction of structural isomers. The differentiation of the ortho (1, 4, 7, 10), meta (2, 5, 8, 11), and para (3, 6, 9, 12) chloro (bromo) substituted in the benzyl substituent isomers of this series of compounds is possible on the basis of comparison of their EI mass spectra coefficients (i.e., ratios of the relative abundances RA of chosen ions) according to the methodology published by us earlier [26, 27]. It should be mentioned that the distinction of ortho (1, 4, 7, 10), meta (2, 5, 8, 11), and para (3, 6, 9, 12) chloro (bromo) substituted isomers of 1–12 is also possible on the basis of the differences in their retention times by gas chromatography.

References


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