Two-Column Gas Chromatography of Trimethylsilyl Derivatives of Biochemically Significant Compounds

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The use of trimethylsilyl (TMS) derivatives to improve the gas chromatographic (GC) properties of biochemically significant compounds has gained wide acceptance. Such derivatives are formed rapidly, conveniently, and with a wide range of compounds. Silylation has been used for the GC analysis of such diverse categories as steroids (1-3), aromatic acids (4-7), nucleic acid components (8-11), amino acids (12-14), carbohydrates (15-17), anions (18-19), amines (4,20), organic acids (21), and vitamins (22). In nearly each case, unique reagents and reaction conditions (solvent, time, and temperature) were employed. In our laboratory it was necessary to establish a single derivatization technique to analyze qualitatively a large number of samples, primarily fractions isolated from human urines, for compounds from several of the above categories. The reagent chosen, bis(trimethylsilyl)trifluoroacetamide (BSTFA) catalyzed with 1% trimethylchlorosilane (TMCS) is one of the most potent silylating agents available; it has the added advantage that its reaction products are highly volatile and elute early along with the solvent and excess reagent (23). Extended heating of the reaction mixture is also used to promote silylation of compounds normally difficult to derivatize. In a very few instances these vigorous conditions lead to the appearance of additional chromatographic peaks that are not present when previously published optimum conditions are employed; this multiplicity of peaks, however, is reproducible and does not interfere with qualitative analyses.

In order to use gas chromatographic reference data for the identification of unknown components, a reproducible method of reporting retention data is necessary. The two most suitable techniques are retention

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indices (24) (RI) and methylene unit (MU) values (4), which differ in magnitude by a factor of 100 (RI 1000 = MU 10.00). These values are obtained by cochromatographing a series of n-alkanes with the sample. When temperature programming is employed, a sufficiently linear relationship exists that a MU value can be calculated by linear interpolation; for example, a compound eluting exactly midway between nonane (C9) and decane (C10) would be assigned an MU value of 9.50. Because the reference hydrocarbons are chromatographed simultaneously with the sample and give peaks closely bracketing those of the sample, minor variations in operating conditions do not affect the retention data, which become primarily a function of the nature of the stationary phase. Methylene unit values are determined on two different stationary phases, OV-1 (nonpolar) and OV-17 (moderately polar), to increase the reliability of identifications based on the GC retention data. In addition to providing an empirical tabulation of data for direct comparison, the data obtained on the two different stationary phases contain several distinct trends in MU values and ΔMU values (the difference between the MU values on OV-17 and OV-1, i.e., ΔMU = MU_{OV-17} - MU_{OV-1}). These trends will be discussed below under "Results and Discussion."

EXPERIMENTAL

Preparation of Derivatives. A 1 mg sample of reference compound is placed in a 3.5 ml septum-capped vial (Schwarz BioResearch). Then 100 μl each of dry pyridine (Pierce Chemical Co.) and BSTFA containing 1% TMCS (Regis Chemical Co.) are added to the vial, thoroughly mixed, and heated for 16 hr at 60°C. This time interval can be decreased, but it was found convenient to heat the samples overnight. All the compounds studied were obtained from commercial sources with the exception of some of the substituted hippuric acids, which were isolated from human urine by anion-exchange chromatography (25).

Gas Chromatography. Aliquots (4 μl) are withdrawn from the above reaction mixtures and injected directly onto the two GC columns of a MicroTek MT-220 gas chromatograph equipped with a dual-flame ionization detector and a dual electrometer. The two columns (6 ft × 0.25 in. o.d. Pyrex tubing packed with 3% OV-1 or 3% OV-17 on 80/100 mesh Chromosorb W-HP) are temperature-programmed simultaneously in the same oven from 100° to 325°C at 10°/min. An initial temperature of 70° is used for compounds with MU values less than 12.0. Helium carrier gas flow rates of 80 ml/min are used with both columns. A series of n-alkanes in hexane is then added to the samples, and the same chromatographic conditions are employed to obtain the data for the calculation of MU values.
RESULTS AND DISCUSSION

The use of more than one column to provide gas chromatographic retention data generally enhances the value of such data. The use of two columns not only increases the probability of the data being unique for a given compound, but also provides some correlation between retention and the structure of the compound being investigated. The two stationary phases in these studies are OV-1 (a methylsilicone gum) and OV-17 (a 50% phenyl/50% methyl silicone gum). The OV-1 is considered a non-polar stationary phase, while the moderately polar OV-17 is somewhat selective, particularly toward aromatic compounds.

Tables 1-7 list the MU values on OV-1 and the \( \Delta MU \) (\( MU_{ov-17} - MU_{ov-1} \)) values for the TMS derivatives of approximately 250 biochemically significant compounds. The tables are categorized into similar chemical types. The retention data within each category are generally understandable in view of a few simple concepts. The familiar rule that "like dissolves like" is a valid guideline when considering the OV-1 and OV-17 stationary phases. The polar phenyl groups in the OV-17 polymer chain make it more retentive toward polar compounds; highly polar compounds are therefore characterized by large positive values for \( \Delta MU \). On the other hand, OV-1, normally considered a nonselective stationary phase, should perhaps be considered selective for highly methylated compounds; the TMS derivative of raffinose, for example, with eleven TMS groups shows a highly negative \( \Delta MU \) value of \(-3.12\). Work presently in progress indicates that negative \( \Delta MU \) values are the general rule for carbohydrates. When employing TMS derivatives with these two stationary phases, the magnitude of \( \Delta MU \) depends largely on how well the TMS groups form a "shield" of methyl groups around the original polar functional groups of the parent compound. This concept is particularly evident among the amino acids and will be discussed below.

**Amino Acids.** As the data in Table 1 indicate, an obvious distinction can be made between the low \( \Delta MU \) values of the aliphatic amino acids and those substituted with a simple hydroxyl group (serine and theonine) or an amino group (ornithine and lysine) and the higher \( \Delta MU \) values for the aromatic amino acids (phenylalanine, tyrosine, histidine, tryptophan, and kynurenine) and highly substituted amino acids (asparagine, glutamine, and citrulline). Within the low \( \Delta MU \) group of amino acids the shielding effect of the TMS groups can be seen. Glycine, \( \beta \)-alanine, \( \beta \)-aminoisobutyric acid, and 4-hydroxylysine all formed two derivatives under the conditions employed in these studies; each of these compounds contains a terminal amino group that is only slightly hindered. This formation of two derivatives is consistent with the work reported by
TABLE 1
MU Values on OV-1 and ΔMU (OV-17) – (OV-1) Values for TMS-Amino Acids

<table>
<thead>
<tr>
<th>Compound</th>
<th>MU_{OV-1}</th>
<th>ΔMU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatic</strong></td>
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<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>11.11</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>13.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Alanine</td>
<td>11.05</td>
<td>0.20</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>11.90</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>14.38</td>
<td>0.02</td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>11.77</td>
<td>0.23</td>
</tr>
<tr>
<td>γ-Aminobutyric acid</td>
<td>15.46</td>
<td>0.11</td>
</tr>
<tr>
<td>α-Aminoisobutyric acid</td>
<td>11.48</td>
<td>0.12</td>
</tr>
<tr>
<td>β-Aminoisobutyric acid</td>
<td>12.16</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>14.74</td>
<td>0.02</td>
</tr>
<tr>
<td>Valine</td>
<td>12.34</td>
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</tr>
<tr>
<td>Leucine</td>
<td>12.84</td>
<td>0.07</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>13.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Sarcosine</td>
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<td>0.30</td>
</tr>
<tr>
<td>Serine</td>
<td>13.80</td>
<td>0.30</td>
</tr>
<tr>
<td>Threonine</td>
<td>14.04</td>
<td>-0.07</td>
</tr>
<tr>
<td><strong>Aromatic and Cyclic</strong></td>
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<td></td>
</tr>
<tr>
<td>Proline</td>
<td>13.02</td>
<td>0.48</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>15.46</td>
<td>0.17</td>
</tr>
<tr>
<td>Piperolic acid</td>
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<td>0.40</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>16.25</td>
<td>0.99</td>
</tr>
<tr>
<td>Tyrosine</td>
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<td>0.67</td>
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<tr>
<td>Histidine</td>
<td>19.14</td>
<td>1.84</td>
</tr>
<tr>
<td>Tryptophan</td>
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<tr>
<td>Kynurenine</td>
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<td>1.71</td>
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<tr>
<td><strong>Sulfer-Containing</strong></td>
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<td></td>
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<td>Cysteine</td>
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<td>0.54</td>
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<td>Cystine</td>
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<td>Homocystine</td>
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<td>Lanthionine</td>
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<td>0.42</td>
</tr>
<tr>
<td>Cystathionine</td>
<td>22.33</td>
<td>0.49</td>
</tr>
<tr>
<td>Methionine</td>
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<td>0.87</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>19.69</td>
<td>0.98</td>
</tr>
<tr>
<td>Djenkolic acid</td>
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<td>1.02</td>
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<tr>
<td><strong>Acidic</strong></td>
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<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>15.41</td>
<td>0.47</td>
</tr>
<tr>
<td>Glutaming acid</td>
<td>16.20</td>
<td>0.60</td>
</tr>
<tr>
<td>Asparagine</td>
<td>16.87</td>
<td>1.13</td>
</tr>
<tr>
<td>Glutamine</td>
<td>17.73</td>
<td>1.14</td>
</tr>
<tr>
<td><strong>Basic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornithine</td>
<td>18.53</td>
<td>-0.32</td>
</tr>
<tr>
<td>Lysine</td>
<td>19.56</td>
<td>-0.30</td>
</tr>
<tr>
<td>Compound</td>
<td>$\text{MU}_{\text{OV-1}}$</td>
<td>$\Delta\text{MU}$</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>4-Hydroxylysine</td>
<td>18.90</td>
<td>-0.35</td>
</tr>
<tr>
<td></td>
<td>21.19</td>
<td>-0.61</td>
</tr>
<tr>
<td>Citrulline</td>
<td>18.36</td>
<td>0.67</td>
</tr>
<tr>
<td>Arginine$^4$</td>
<td>16.32</td>
<td>0.08</td>
</tr>
</tbody>
</table>

$^1$ See Table 5 for other phenylalanine derivatives.

$^2$ See Table 7 for other histidine derivatives; second peak on OV-1, $\text{MU} = 18.00$.

$^3$ See Table 7 for other tryptophan derivatives.

$^4$ Secondary peaks on OV-17.

Bergstrom et al. (13), who reported two such derivatives for glycine and lysine under different derivatization conditions. Using combined gas chromatography-mass spectrometry, they determined that the earlier eluting of the two derivatives was monosilylated on the terminal amino group and the later eluting was disilylated; lysine was observed to convert more readily to the di-TMS-amino than was glycine. The diderivatives have higher $\text{MU}_{\text{OV-1}}$ values in every case because of the higher molecular weight; however, because the two TMS groups more effectively shield the amino group; a smaller $\Delta\text{MU}$ value is observed. Sarcosine, which has a methyl group on the terminal amino group of glycine and can accept only one TMS group, falls between the two glycine derivatives with respect to $\text{MU}_{\text{OV-1}}$ and $\Delta\text{MU}$ as would be predicted. The derivatization conditions used here are apparently more vigorous than those used by Bergström because of the complete conversion of lysine to the di-TMS-amino derivative. Ornithine and $\gamma$-aminobutyric acid, which like lysine have an essentially unhindered terminal amino group, also form the diderivative exclusively, as evidenced by a high $\text{MU}_{\text{OV-1}}$ value and a low $\Delta\text{MU}$. This concept is further illustrated by 4-hydroxylysine, the terminal amino group of which is partially hindered by the hydroxyl group, thereby forming two derivatives. Again the di-TMS-amino derivative has a larger $\text{MU}$ and a smaller $\Delta\text{MU}$. The $\Delta\text{MU}$ for penta-TMS-4-hydroxylysine is lower than that for tetra-TMS-lysine because the hydroxyl group is well shielded by the TMS group and its secondary position in the amino acid, and the additional TMS group increases the methylated nature of the compound. The $\text{MU}_{\text{OV-1}}$ value for the 4-hydroxy derivative is larger because of its higher molecular weight. The influence of a hydroxyl group located in a secondary position versus a primary position where it is not as well shielded is seen by comparing theonine with a secondary hydroxyl group and a $\Delta\text{MU}$ of -0.07 to serine with a primary hydroxyl and a $\Delta\text{MU}$ of 0.30. The effect of additional hydroxyl groups is to increase the $\text{MU}_{\text{OV-1}}$ value and decrease the $\Delta\text{MU}$, for
example: proline (13.02, 0.48) and hydroxyproline (15.48, 0.17); phenylalanine (16.25, 0.99), 4-hydroxyphenylalanine (tyrosine; 19.52, 0.67), and 3,4-dihydroxyphenylalanine (Table 5; 21.24, 0.39).

Organic Acids. The data in Table 2 show a regular increase in $MU_{ov-1}$ for the homologous diacids (oxalic, malonic, succinic, glutaric, and adipic) and a constant value of $\Delta MU$ for all except the first member of the series. This is typical behavior for homologs, as seen throughout Tables 1-7. As with the amino acids, additional silylated hydroxyl groups increase the methylated nature of the organic acids, increasing $MU_{ov-1}$ and decreasing $\Delta MU$. Evidence for this is seen with succinic acid and its hydroxyderivatives: succinic acid (13.23, 0.94), malic acid (15.00, 0.54), and tartaric acid (16.80, 0.39).

Aromatic Acids, Amines, and Glycine Conjugates. The aromatic acids

<table>
<thead>
<tr>
<th>Compound</th>
<th>$MU_{ov-1}$</th>
<th>$\Delta MU$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diacids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>11.14</td>
<td>1.40</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>12.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Methylmalonic acid</td>
<td>12.13</td>
<td>0.98</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>13.23</td>
<td>0.94</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>13.52</td>
<td>0.57</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>14.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>15.02</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Triacid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetoinic acid (trans)</td>
<td>17.56</td>
<td>1.03</td>
</tr>
<tr>
<td><strong>Hydroxy Acids</strong></td>
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<td></td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>10.45</td>
<td>0.68</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>10.65</td>
<td>0.50</td>
</tr>
<tr>
<td>Malic acid</td>
<td>15.00</td>
<td>0.54</td>
</tr>
<tr>
<td>Oxalacetic acid</td>
<td>15.65</td>
<td>0.74</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>16.80</td>
<td>0.39</td>
</tr>
<tr>
<td>Citric acid</td>
<td>18.57</td>
<td>0.45</td>
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<tr>
<td><strong>$\alpha$-Keto Acids</strong></td>
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<tr>
<td>Glyoxylic acid</td>
<td>12.65</td>
<td>0.28</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>10.91</td>
<td>0.40</td>
</tr>
<tr>
<td>$\alpha$-Ketobutyric acid</td>
<td>11.79</td>
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<td>$\alpha$-Ketovaleric acid</td>
<td>12.39</td>
<td>0.52</td>
</tr>
<tr>
<td>$\alpha$-Ketoglutaric acid</td>
<td>18.67</td>
<td>0.75</td>
</tr>
</tbody>
</table>

1 Secondary peak on both columns, $MU = 14.32$ (OV-1) and 15.20 (OV-17), $\Delta MU = 0.88$. 

TABLE 2
MU Values on OV-1 and $\Delta MU$ (OV-17) − (OV-1) Values for TMS-Organic Acids
**TABLE 3**

MU Values on OV-1 and ΔMU (OV-17) — (OV-1) Values for TMS-Aromatic Acids

(ΔMU values are shown below corresponding MU values)

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Benzolic</th>
<th>Phenylacetic</th>
<th>Phenylproionic</th>
<th>Mandelic</th>
<th>Phenylmandelic</th>
<th>Cinnamic</th>
<th>Phenylcinnamic</th>
<th>Phenylpyruvic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unsubstituted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>12.28</td>
<td>12.75</td>
<td>13.97</td>
<td>14.77</td>
<td>15.82</td>
<td>15.24</td>
<td>17.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.35</td>
<td>1.64</td>
<td>1.64</td>
<td>1.18</td>
<td>1.11</td>
<td>1.89</td>
<td>1.26</td>
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</tr>
<tr>
<td><strong>2-Methoxy</strong></td>
<td>14.31</td>
<td>14.76</td>
<td>15.95</td>
<td>16.18</td>
<td></td>
<td>17.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.23</td>
<td>2.08</td>
<td>2.08</td>
<td>1.82</td>
<td></td>
<td>2.57</td>
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<td></td>
</tr>
<tr>
<td><strong>3-Methoxy</strong></td>
<td>14.55</td>
<td>14.96</td>
<td></td>
<td>16.60</td>
<td>17.67</td>
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<tr>
<td></td>
<td>1.86</td>
<td>2.10</td>
<td></td>
<td>1.77</td>
<td></td>
<td>2.30</td>
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<tr>
<td><strong>4-Methoxy</strong></td>
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<td>15.21</td>
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<td>16.97</td>
<td>17.93</td>
<td>18.06</td>
<td>19.53</td>
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<tr>
<td></td>
<td>1.98</td>
<td>2.14</td>
<td>2.11</td>
<td>1.76</td>
<td>1.66</td>
<td>2.58</td>
<td>1.90</td>
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<tr>
<td><strong>2-Hydroxy</strong></td>
<td>15.10</td>
<td>15.65</td>
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<td></td>
<td></td>
<td>18.00</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1.17</td>
<td>1.30</td>
<td></td>
<td></td>
<td></td>
<td>1.63</td>
<td></td>
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</tr>
<tr>
<td><strong>3-Hydroxy</strong></td>
<td>15.55</td>
<td>16.00</td>
<td>17.17</td>
<td>17.48</td>
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<td>18.64</td>
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</tr>
<tr>
<td></td>
<td>1.35</td>
<td>1.56</td>
<td>1.31</td>
<td>0.92</td>
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<td></td>
<td>1.12</td>
<td>1.39</td>
<td>1.35</td>
<td>1.04</td>
<td>0.88</td>
<td>1.62</td>
<td>0.95</td>
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<td><strong>3,4-Dimethoxy</strong></td>
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<td>2.67</td>
<td>2.77</td>
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<tr>
<td><strong>3-Hydroxy-4-methoxy</strong></td>
<td>17.61</td>
<td>17.57</td>
<td>18.72</td>
<td>18.94</td>
<td></td>
<td>20.63</td>
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<td></td>
<td>1.68</td>
<td>1.77</td>
<td>1.75</td>
<td>1.22</td>
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<td>1.94</td>
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<td><strong>3-Methoxy-4-hydroxy</strong></td>
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<td>17.58</td>
<td>18.84</td>
<td>18.90</td>
<td>20.22</td>
<td>20.76</td>
<td>21.65</td>
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<td></td>
<td>1.53</td>
<td>1.78</td>
<td>1.69</td>
<td>1.34</td>
<td>1.15</td>
<td>1.98</td>
<td>1.65</td>
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<td><strong>2,3-Dihydroxy</strong></td>
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</table>
are the largest group of closely related compounds involved in this study and nearly all of the data fit into a definite pattern. Table 3 contains the $MU_{ov-1}$ and $\Delta MU$ values for 67 aromatic acids. Two trends are immediately evident: regardless of substitution, the $MU_{ov-1}$ values increase in the order benzoic < phenylacetic < phenylpropionic < mandelic < phenyllactic < cinnamic < phenylpyruvic; similarly, regardless of the parent acid, substitution affects $MU_{ov-1}$ in the order unsubstituted < methoxy < hydroxy < dimethoxy < hydroxy, methoxy < dihydroxy. Within subgroups of monosubstituted compounds (methoxy, hydroxy, or amino), the position of substitution affects the $MU_{ov-1}$ value in the order ortho < meta < para. The glycine conjugates (Table 4) and aromatic amines (Table 5) also reflect these trends in $MU_{ov-1}$ as a function of substitution.

Equally regular data are observed among the $\Delta MU$ values for these aromatic compounds, although the orders of dependence are different from those for the $MU_{ov-1}$ values. The $\Delta MU$ values increase with the parent acid, regardless of substitution, in the order phenyllactic < mandelic < phenylpyruvic < benzoic < phenylpropionic < phenylacetic < cinnamic. The TMS-derivatives of phenyllactic and mandelic acids have the lowest values of $\Delta MU$ because they contain a well-shielded TMS-hydroxyl group. The low $\Delta MU$ (and high $MU_{ov-1}$) values indicate that phenylpyruvic acid and its substituted forms have enolized and the resulting hydroxyl group is silylated. The cinnamic acids have the largest $\Delta MU$ values because of the polar alkene group. Substitution also affects

### TABLE 4
MU Values on OV-1 and $\Delta MU$ (OV-17) -- (OV-1) Values for TMS Derivatives of Glycine Conjugates of Aromatic Acids

<table>
<thead>
<tr>
<th>Compound</th>
<th>$MU_{ov-1}$</th>
<th>$MU_{ov-17}$</th>
<th>$\Delta MU$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>19.91</td>
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<td>21.08</td>
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<td>19.54</td>
<td>31.06</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>20.46</td>
<td>22.95</td>
<td>2.49</td>
</tr>
<tr>
<td>3-Hydroxyhippuric acid</td>
<td>20.55</td>
<td>22.20</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>21.21</td>
<td>23.78</td>
<td>2.57</td>
</tr>
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<td>4-Hydroxyhippuric acid</td>
<td>21.22</td>
<td>22.83</td>
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</tr>
<tr>
<td></td>
<td>21.92</td>
<td>24.56</td>
<td>2.64</td>
</tr>
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<td>22.87</td>
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<tr>
<td></td>
<td>20.77</td>
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<td>3.74</td>
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<td>23.00</td>
<td>25.23</td>
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<tr>
<td></td>
<td>24.00</td>
<td>27.36</td>
<td>3.36</td>
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<td>Nicotinuric acid</td>
<td>18.48</td>
<td>20.71</td>
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<td></td>
<td></td>
<td>21.69</td>
<td>3.21</td>
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</table>
## TABLE 5

MU Values on OV-1 and ΔMU (OV-17) – (OV-1) Values for TMS-Aromatic Amines

<table>
<thead>
<tr>
<th>Compound</th>
<th>MU&lt;sub&gt;OV-1&lt;/sub&gt;</th>
<th>ΔMU</th>
</tr>
</thead>
<tbody>
<tr>
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<td>15.68</td>
<td>0.63</td>
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<tr>
<td>Tyramine</td>
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<td>0.37</td>
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<td>Dopamine</td>
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<td>0.08</td>
</tr>
<tr>
<td>3-Methoxytyramine</td>
<td>20.48</td>
<td>0.68</td>
</tr>
<tr>
<td>3,4-Dimethoxyphenylethylamine</td>
<td>19.78</td>
<td>1.62</td>
</tr>
<tr>
<td>Epinephrine</td>
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</tr>
<tr>
<td>Metanephrine</td>
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<td>0.44</td>
</tr>
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<td>Norepinephrine</td>
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<tr>
<td>Normetanephrine</td>
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<td>0.59</td>
</tr>
<tr>
<td>Phenylalanine</td>
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</tr>
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<td>N-Acetylphenylalanine</td>
<td>17.90</td>
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</tr>
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<td>Tyrosine</td>
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<td>0.67</td>
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<td>3,4-Dihydroxyphenylalanine (dopa)</td>
<td>21.24</td>
<td>0.39</td>
</tr>
</tbody>
</table>

ΔMU differently than MU<sub>OV-1</sub>; ΔMU values increase in the order dihydroxy < hydroxy < unsubstituted < methoxy, hydroxy < methoxy < dimethoxy. This trend occurs because the hydroxy groups are silylated, forming highly methylated compounds, whereas the methoxyl groups are not silylated and remain relatively polar. The silylated hydroxyl groups therefore reduce ΔMU and the polar methoxyl groups increase it. This same trend also exists for the aromatic amines and glycine conjugates.

A characteristic unique to the glycine conjugates (Table 4) is that, under the conditions employed in these studies, they consistently form two GC peaks with a relatively constant difference in ΔMU values of approximately 1.1. The two peaks are most likely attributable to the amide group in these compounds being partially enolized. The hydroxyl group of the enol form is silylated and, because of this increased methylated nature, it can probably be assigned to the peak with the smaller ΔMU, which is the earlier eluting peak in every instance.

**Purines, Pyrimidines, and Other Nitrogen-Heterocyclic Compounds.** It is significantly more difficult to relate MU<sub>OV-1</sub> and ΔMU values to the structure of the nitrogen-heterocycles than it is for the compounds previously discussed. The complex nature of the heterocyclic rings enable several influences to be operating simultaneously. The aromatic and polar nature of these compounds is reflected in the generally high ΔMU values found in Tables 6 and 7. However as seen in the data in these tables the relationships discussed above, particularly for hydroxyl and methyl groups, also exist for closely related nitrogen-heterocyclic compounds. Caffeine (1,3,7-trimethylxanthine) has the largest ΔMU of any compound in Tables 1–7 and it is the only compound listed that is not
TABLE 6
MU Values on OV-1 and ΔMU (OV-17) - (OV-1) Values for TMS-Purines and Pyrimidines Including Nucleosides

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<thead>
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<th>Parent base</th>
<th>Base</th>
<th>MU0V-1</th>
<th>ΔMU</th>
<th>Deoxyribose</th>
<th>MU0V-1</th>
<th>ΔMU</th>
<th>Ribose</th>
<th>MU0V-1</th>
<th>ΔMU</th>
</tr>
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<tbody>
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<td><strong>Pyrimidines</strong></td>
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<tr>
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<td>24.60</td>
<td>1.85</td>
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<td>26.90</td>
<td>0.98</td>
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<tr>
<td>7-Methylxanthine</td>
<td>20.16</td>
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<td>1-Methylxanthine</td>
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<td>3.43</td>
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<td>3,7-Dimethylxanthine</td>
<td>21.13</td>
<td>2.24</td>
<td></td>
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<tr>
<td>2,6-Dithioxanthine</td>
<td>24.66</td>
<td>3.54</td>
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<tr>
<td>Uric acid</td>
<td>21.13</td>
<td>1.46</td>
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</tr>
</tbody>
</table>

1 Additional large peak on OV-1, MU = 27.95.
2 Additional large peak on OV-17, MU = 32.4.
TABLE 7
MU Values on OV-1 and ΔMU (OV-17) – (OV-1) Values for TMS Derivatives of Nitrogen-Heterocyclic Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>MU&lt;sub&gt;OV-1&lt;/sub&gt;</th>
<th>ΔMU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyridines</strong></td>
<td></td>
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</tr>
<tr>
<td>Picolinic acid</td>
<td>12.88</td>
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</tr>
<tr>
<td>Nicotinic acid</td>
<td>12.74</td>
<td>1.58</td>
</tr>
<tr>
<td>Nicotinuric acid&lt;sup&gt;1&lt;/sup&gt;</td>
<td>18.48</td>
<td>2.23</td>
</tr>
<tr>
<td>Quinolinic acid</td>
<td>17.08</td>
<td>2.58</td>
</tr>
<tr>
<td>Nicotinamide&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14.58</td>
<td>2.37</td>
</tr>
<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;-Methylnicotinamide&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14.31</td>
<td>1.99</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>17.45</td>
<td>1.55</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>19.08</td>
<td>0.92</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>19.48</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Quinolines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Hydroxyquinoline</td>
<td>16.00</td>
<td>2.23</td>
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<tr>
<td>Quinaldic acid</td>
<td>17.57</td>
<td>3.14</td>
</tr>
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<td>Kynurenic acid</td>
<td>20.59</td>
<td>2.50</td>
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<td>Xanthenic acid</td>
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<td><strong>Imidazoles</strong></td>
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<td>3.05</td>
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<td>Urocanic acid</td>
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<td>3.62</td>
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<tr>
<td>Imidazole-5-lactic acid</td>
<td>17.87</td>
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</tr>
<tr>
<td>Hydantoin-5-acetic acid</td>
<td>18.00</td>
<td>1.57</td>
</tr>
<tr>
<td>Imidazole-4,5-dicarboxylic acid</td>
<td>18.95</td>
<td>2.28</td>
</tr>
<tr>
<td>Histamine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>18.73</td>
<td>1.58</td>
</tr>
<tr>
<td>1-Methylhistamine&lt;sup&gt;1&lt;/sup&gt;</td>
<td>17.32</td>
<td>2.19</td>
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<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;-Acetylhistamine</td>
<td>18.09</td>
<td>2.51</td>
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<tr>
<td>Histidine&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>1-Methylhistidine</td>
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<td>17.35</td>
<td>2.24</td>
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<td>2-Thiohistidine</td>
<td>22.51</td>
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<tr>
<td>4-Aminimidazole-5-carboxamide</td>
<td>18.11</td>
<td>0.60</td>
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<tr>
<td>Allantoin&lt;sup&gt;3&lt;/sup&gt;</td>
<td>19.69</td>
<td>0.98</td>
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<tr>
<td><strong>Indoles</strong></td>
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<tr>
<td>Indole-3-carboxylic acid</td>
<td>19.99</td>
<td>2.64</td>
</tr>
<tr>
<td>Indole-3-acetic acid</td>
<td>19.38</td>
<td>1.55</td>
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<td>Indole-3-propionic acid</td>
<td>20.66</td>
<td>2.40</td>
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<tr>
<td>Indole-3-glycollie acid</td>
<td>20.18</td>
<td>2.31</td>
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<tr>
<td>Indole-3-lactic acid</td>
<td>21.80</td>
<td>1.76</td>
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<tr>
<td>Indole-3-acrylic acid</td>
<td>23.57</td>
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<td>Indole-3-pyruvic acid</td>
<td>24.33</td>
<td>2.02</td>
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<tr>
<td>5-Hydroxyindole-3-acetic acid</td>
<td>22.04</td>
<td>2.36</td>
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<td>Indole-3-acetamide&lt;sup&gt;1&lt;/sup&gt;</td>
<td>20.66</td>
<td>3.16</td>
</tr>
<tr>
<td>5-Hydroxytryptophol</td>
<td>21.56</td>
<td>1.63</td>
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<tr>
<td>5-Methoxytryptophol</td>
<td>20.92</td>
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<tr>
<td>Tryptamine</td>
<td>22.19</td>
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<td>Serotonin</td>
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<td>Melatonin</td>
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<tr>
<td>Bufotenin</td>
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</tr>
<tr>
<td>Tryptophan</td>
<td>22.15</td>
<td>1.69</td>
</tr>
<tr>
<td>5-Hydroxytryptophan</td>
<td>24.60</td>
<td>1.41</td>
</tr>
<tr>
<td>N&lt;sup&gt;1&lt;/sup&gt;-Acetyltryptophan&lt;sup&gt;1&lt;/sup&gt;</td>
<td>24.18</td>
<td>1.82</td>
</tr>
<tr>
<td>Indican</td>
<td>16.91</td>
<td>1.78</td>
</tr>
</tbody>
</table>

<sup>1</sup> Secondary peak on both columns.
<sup>2</sup> Secondary peak on OV-1, MU = 18.00.
silylated, the methyl groups being present in the positions at which xanthine would silylate.

The data in Table 6 show that MU_{ov-1} values increase in the order base < deoxyriboside < riboside for both purines and pyrimidines. Previously published work (11) indicates the ribonucleosides are followed by 2'-nucleotides ≅ 3'-nucleotides < 5'-nucleotides. The ΔMU values are consistently lower for the deoxyribosides compared to the ribosides; this is consistent with the effect of hydroxyl groups discussed earlier.

By using these types of GC data in conjunction with other analytical methods, more than 70 urinary constituents, including compounds from all of the categories discussed in this manuscript, have been identified (25). Although the tables of data in this manuscript are useful for structure correlations and investigation of particular categories, a single listing of all the data (26) arranged by increasing MU_{ov-1} has been found the most useful for the identification of unknown compounds. These data have been put on punch cards and a FORTRAN IV computer program for an IBM 360 has been written to facilitate storage and searching of the data.

SUMMARY

Gas chromatographic (GC) retention data as methylene unit (MU) values are presented for the trimethylsilyl (TMS) derivatives of 250 biochemically significant compounds on two different GC stationary phases (OV-1 and OV-17). A ΔMU value is calculated for the difference in MU values on the moderately polar OV-17 and the nonpolar OV-1 columns. Some relationships between ΔMU values and structure are discussed; in general highly polar compounds have large ΔMU values and those compounds forming derivatives containing several TMS groups have low ΔMU values. Compounds in the following categories were studied: amino acids; organic acids; aromatic acids, amines, and glycine conjugates; and purines, pyrimidines, and other nitrogen heterocyclic compounds.

REFERENCES
10. R. L. Hancock, J. Gas Chromatogr. 6, 431 (1968).