New Approach to Derivatization and Gas-Chromatographic Analysis of Barbiturates

Richard H. Greeley

A recently developed procedure for alkylation of organic acids has proven extremely successful for preparation of volatile alkyl derivatives of the barbiturates for gas-chromatographic analysis. Soluble salts are formed in a mixture of N,N-dimethylacetamide and methanol. These in turn react rapidly with alkyl iodides to form the corresponding alkyl derivatives. The butyl derivatives of barbiturates, prepared in this manner, are separable by high-resolution gas-chromatography. Any of 14 barbiturates can be determined simultaneously or separately (although there is some overlap with certain uncommon barbiturates). The butyl derivatives of several barbiturates that are unresolved in the form of undervived compounds or methyl esters can be resolved, thus overcoming many previous analytical limitations.

A number of papers (1–3) have recently been published on the preparation of alkyl derivatives of the barbiturates for gas-chromatographic analysis by pyrolysis of their quaternary amine salts on the column. It is generally acknowledged that, other factors being equal, the alkyl derivatives of barbiturates can usually be analyzed by gas chromatography with less peak-tailing, less irreversible adsorption on the column, and, therefore, greater accuracy and reproducibility than is the case for underivatized compounds.

However, the reagents for derivatization are rapidly deleterious to gas-chromatographic columns, so the initial advantage of these derivatization methods is short-lived. Moreover, pyrolysis techniques are limited, at present, to preparation of methyl and ethyl derivatives. Substitution of a new derivatization procedure allows preparation of the butyl derivatives of barbiturates, and use of a high-resolution column allows 14 sedative drugs to be well separated simultaneously, without the use of reagents deleterious to gas-chromatographic columns.

Materials and Methods

Gas-chromatographic analyses were done with a Model 402 gas chromatograph with flame detector (Hewlett Packard Co., Palo Alto, Calif. 94303). A 180 cm by 2 mm (i.d.) glass column packed with “2% OV-17 on Chromosorb W-HP 80/100” (Supelco, Inc., Bellefonte, Pa. 16823) was used in preliminary work, while a 180 cm by 2 mm (i.d.) glass column packed with “1½% SP-2250 on Super W” (Pacific Analytical Consultants, Palo Alto, Calif. 94303) and rated at 5000 theoretical plates for isothermal work was used in the differential gas-chromatographic analysis of the barbiturates. The columns were treated by injection of “Silyl 8” (Pierce Chemical Co., Rockford, Ill. 61105) at 200 °C after every 8–10 analytical injections to prevent any loss in performance. Columns were ready for reuse after 10 min after treatment.

N,N-dimethylacetamide (“spectroquality”), methanol (“spectroquality”), iodomethane, and tetramethylammonium hydroxide in methanol (24:76 by vol) were obtained from Matheson Scientific. Phenyltrimethylammonium hydroxide in methanol (0.1 mol/liter), iodoethane, 1-iodobutane, and 2-iodobutane were obtained from Eastman Kodak Co., Rochester, N. Y. 14850. Barbiturates were obtained as unadulterated free compounds or salts from our hospital pharmacy.

Derivatization of Phenobarbital with Phenyltrimethylammonium Hydroxide as Base

Add 1.0 ml of the phenyltrimethylammonium hydroxide–methanol mixture (100 μmol) and 4.0 ml of N,N-dimethylacetamide to 5.0 mg of phenobarbital (21.5 μmol) and agitate gently.

After the phenobarbital has dissolved, add 100 μl of 1-iodobutane (880 μmol) and mix thoroughly. This quantity, which represents a great excess, was selected only for convenience of measurement.

Gas-chromatographic analysis for unreacted starting material, side products, and the butyl derivative shows that 98.3% of the starting material has been
Table 1. Derivatization of Phenobarbital with Phenyltrimethylammonium Hydroxide or Tetramethylammonium Hydroxide as Base

<table>
<thead>
<tr>
<th>Iodide</th>
<th>Yield, %</th>
<th>Reaction time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyltrimethylammonium hydroxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Ethyl</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>1-Butyl</td>
<td>98.3</td>
<td>9</td>
</tr>
<tr>
<td>2-Butyl</td>
<td>multiple products</td>
<td></td>
</tr>
<tr>
<td>Tetramethylammonium hydroxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Ethyl</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>1-Butyl</td>
<td>99.6</td>
<td>3</td>
</tr>
<tr>
<td>2-Butyl</td>
<td>multiple products</td>
<td></td>
</tr>
</tbody>
</table>

converted to the butyl derivative after 9 min (see Table 1 for data on other derivatives).

Derivatization of Phenobarbital with Tetramethylammonium Hydroxide as Base

Add a solution of 50 μl of the tetramethylammonium hydroxide in methanol (104 μmol), 0.96 ml of methanol, and 4.00 ml of N,N-dimethylacetamide to 5.0 mg of phenobarbital (21.5 μmol) and agitate gently. Then add 100 μl of 1-iodobutane and mix thoroughly (tetramethylammonium iodide precipitates slowly). Gas-chromatographic analysis, as above, shows 99.6% conversion to the butyl derivative after 3 min (see Table 1 for data on other derivatives).

Analysis of an Extract of Whole Blood

To a concentrate of the extract of 2 ml of whole blood, prepared according to the extraction procedure of Sunshine (5), add 40 μl of N,N-dimethylacetamide, 5 μl of the tetramethylammonium hydroxide: methanol solution (24:76 by vol), and 10 μl of 1-iodobutane, in that order. Analysis by gas-chromatography is made with temperature programming at 7.5 °C per min from 170 to 260 °C after 4 min isothermal at 170 °C, on a 180 cm ½% SP-2250 on Super W column.

Results and Discussion

A new technique for preparing alkyl derivatives of organic acids, which is particularly applicable to the derivatization of acids for gas-chromatographic analysis, has been extended here to the derivatization of barbiturates with outstanding success.

Gas-chromatographic analysis of the alkyl derivatives of the barbiturates indicates that the reaction is both clean and reproducible. For example, quantitative analysis of several different derivatives of phenobarbital (see Table 1) showed that more than 98% was converted to the alkyl derivative, regardless of the primary iodide or base used.

In short, the technique requires that an acidic compound such as a barbiturate be converted to a soluble salt with an organic base such as phenyltrimethylammonium hydroxide or tetramethylammonium hydroxide in a highly polar solvent system such as methanolic N,N-dimethylacetamide.

The soluble salt thus formed then reacts with an excess of a primary alkyl iodide, resulting in formation of the corresponding alkyl derivative within 10 min. Tetramethylammonium hydroxide, because of the higher concentration of the commercial reagent and its greater stability (4), appears to be the base of choice.

The reagents used by other authors for preparation of alkyl derivatives of the barbiturates by pyrolysis are extremely deleterious to gas-chromatographic columns: they tend to strip the liquid phase from the solid support and to react with the liquid phase and solid support. Usually, loss of linear column response and significant tailing of the eluted peaks occurs after only a few injections. Moreover, the recommended injection port temperatures of 300–350 °C for pyrolysis (3) cause rapid deterioration of the column packing near the injection port.

On the other hand, in the method described here, much less methanol is used in the reagent, resulting in increased column life. Second, since the derivative is formed before the solution is injected, there is no need for a high injection-port temperature. Finally, the destructive quaternary ammonium salts precipitate during the derivatization and are not injected.

Any primary alkyl derivative of the barbiturates can be formed readily, in contrast to the present limitations of the pyrolysis procedure to methyl and ethyl derivatives. This choice of several derivatives allows a much greater latitude in selection of analysis conditions than was previously possible and allows some of the interferences that occasionally occur in physiological systems to be circumvented.

A number of alkyl derivatives were examined to determine the most suitable alkyl derivative for clinical analysis. Good separations of several barbiturates are obtained with the methyl derivatives, but some of the barbiturates used in medicine are already methyl derivatives of other barbiturates, complicating identification, and some of the separations are inadequate. Ethyl derivatives produce less acceptable separations of several barbiturates than the methyl derivatives, although better separations of others.

It appears that the butyl derivatives of the barbiturates give the best overall separation for determinations in blood (applicability for determinations in urine was not investigated), because virtually all of the clinically significant barbiturates can be identified and quantitatively analyzed during a 14-min gas-chromatographic run. Several others are also separated that can be used as internal standards.

When a 180 cm ½% SP-2250/Super W column capable of isothermal resolution of 5000 theoretical plates was used, the butyl derivatives of 14 barbiturates were separated and quantitatively analyzed in the presence of each other. The use of a high-effi-
ciency column such as this is very important in obtaining good separations (Figure 1).

Moreover, no interferences from other components of whole blood were observed, when the barbiturates were isolated by the well-tested back-extraction procedure of Sunshine (5). A final advantage of butyl derivatives is that the detector response for phenobarbitals, which is usually low with other derivatives, was significantly improved by use of the butyl derivative.

Retention times and relative peak heights of 16 barbiturates are listed in Table 2, along with comments on their resolution. Coefficients of variation of the peak heights of the individual barbiturates with respect to separately added internal standard average 3%. These data were obtained by gas-chromatography of about 0.4 µg of each barbiturate along with 2.0 µg of internal standard in each injection; comparable results were obtained when greater or lesser amounts of barbiturates were used with the same amount of internal standard.

Linearity of response is maintained for all the barbiturates with injections of 0.02–4.00 µg. This corresponds to the injection of 1 µL of the derivatization mixture (50 µL total volume) prepared as described from 2 mL of whole blood containing 0.05–10.0 mg of barbiturate per deciliter.

Except for the following cases, peaks overlap by less than 5% of their peak height. Secobarbital and vinylbarbitals (rarely used) are coincident, as in most other systems. Aprobarbital and butalbital have a 38% overlap, and butabarbitals and amobarbitals have a 22% overlap. It should be noted that in the absence of tailing, an overlap as great as 60% will cause only a 1% change in peak height, and peak height was found to be reliable for quantitation here. Butalbital (rarely used) cannot be quantitatively analyzed in the presence of butalbital (rarely used), but either can be identified when it is separately present.

I thank George Gotti and Hans Loken for assistance and advice. This work was supported by an NIH postdoctoral traineeship.

References