Thermal Decomposition of 1,3-Dimethyl Derivative of Phenobarbital in Trimethylanilinium Hydroxide

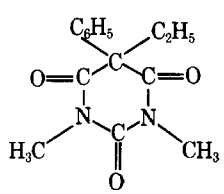
PATRICK S. CALLERY and JAMES LESLIE ˣ

Abstract □ Extensive degradation of the 1,3-dimethyl derivative of phenobarbital occurs when it is injected with trimethylanilinium hydroxide in methanol into a gas chromatograph. The application of mass spectrometry demonstrated that the major products are *N*-methyl-2-phenylbutyramide, *N,N*-dimethyl-2-phenylbutyramide, methyl 2-phenylbutyrate, *N,N,N'*-trimethylethylphenylmalondiamide, and *N,N,N',N'*-tetramethylethylphenylmalondiamide. The methyl group of the ester arises from the methanol solvent, whereas the methyl groups of the substituted amides arise from the parent compound or the methylating reagent. Tetramethylammonium hydroxide causes much more degradation than trimethylanilinium hydroxide under comparable conditions.

Keyphrases □ 1,3-Dimethyl derivative of phenobarbital—thermal decomposition in gas chromatograph, major products identified by mass spectrometry □ Phenobarbital 1,3-dimethyl derivative—thermal decomposition in gas chromatograph, major products identified by mass spectrometry □ Decomposition, thermal—1,3-dimethyl derivative of phenobarbital, in gas chromatograph, major products identified by mass spectrometry □ GLC–mass spectrometry—thermal decomposition of 1,3-dimethyl derivative of phenobarbital, major products identified

Phenobarbital is frequently analyzed by a GC technique in which the drug is injected simultaneously with trimethylanilinium hydroxide (I) (1). The peak quantified was shown to be the 1,3-dimethyl derivative of phenobarbital (II) (2), but the presence of an additional peak with a much shorter retention time was consistently reported (3–7). This "early peak" was identified as *N*-methyl-2-phenylbutyramide (V) (5), although the presence of *N,N*-dimethyl-2-phenylbutyramide (VI) also was demonstrated (7).

In preliminary studies, a mixture of I and II was injected into a gas chromatograph under the conditions of phenobarbital analysis, and it was observed that II degraded to several products (8). In view of the extensive use of the on-column GC methylation procedure for phenobarbital determination and the interest in the structure of the early peak, the products of the reaction of I and II were examined by mass spectrometry; the results are reported in this paper.



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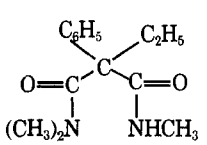

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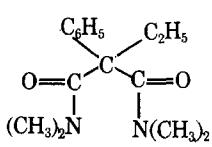

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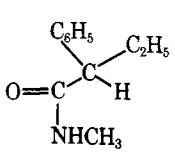

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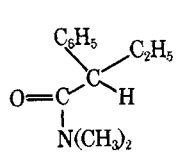

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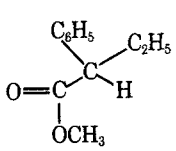

VII

RESULTS

Injection of II in methanol into a gas chromatograph[1] yielded a single peak, whereas injection of this compound in I (0.1–1.8 *M*) at temperatures usually used in the analysis of phenobarbital yielded several peaks (Fig. 1). Peaks A and B had the same retention times as the early peak and major peak, respectively, observed when phenobarbital is injected with I under the same conditions. Peaks C and D were not reported previously.

Furthermore, analysis of the mixture of I and II at a programmed temperature starting at 90° revealed the presence of two additional components, E and F, and resolved peak A into two peaks, A_1 and A_2 (Fig. 2*a*). The relative areas of the peaks were dependent on a number of factors, including the absolute and relative concentrations of I and II, the water content of the mixture injected, and the pH of the solution of I used. By comparison of their mass spectra to those obtained for authentic compounds, the identities of peaks A_1, A_2, B, E, and F were established as VI, V, II, dimethylaniline, and methyl 2-phenylbutyrate (VII), respectively.

The mass spectra of peaks C and D (Figs. 3 and 4) indicated that these compounds were *N,N,N'*-trimethylphenylethylmalondiamide (III) and *N,N,N',N'*-tetramethylphenylethylmalondiamide (IV), respectively. The fragmentation pattern of peak D corresponded closely to the literature values for IV (9). Peak C was assigned Structure III on the basis of

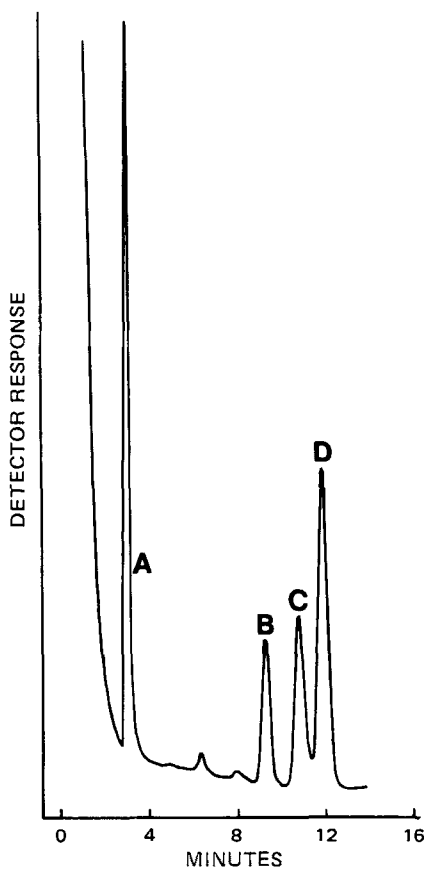

Figure 1—*Gas chromatogram of a mixture of I and II isothermally at 220°.*

[1] Varian series 1400.

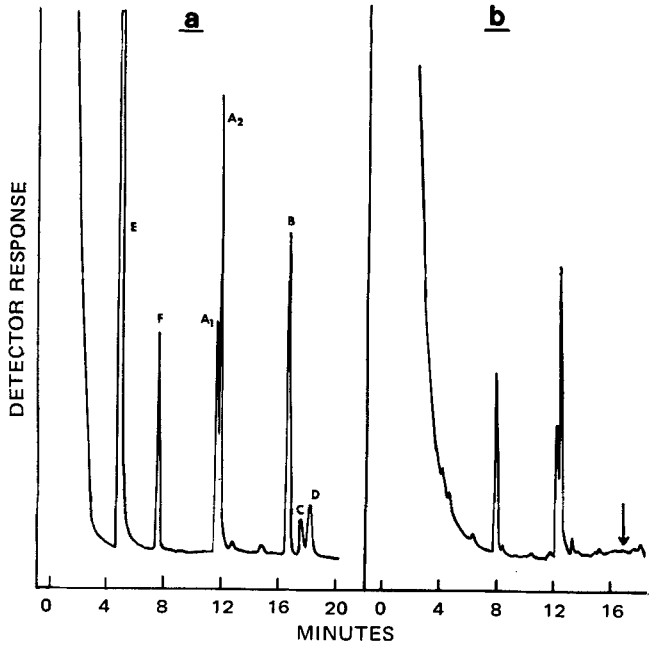

Figure 2—(a) *Gas chromatogram of a mixture of I and II programmed from 90 to 260° at 10°/min.* (b) *Gas chromatogram of a mixture of II and tetramethylammonium hydroxide programmed from 90 to 260° at 10°/min. The arrow indicates where unchanged II would have appeared.*

an analysis of its fragmentation pattern. Peak C exhibited significant ions also found for IV at *m/e* 120, 117, 103, 91, and 72, which suggested that C and D were derived from a similar skeleton structure.

The molecular ion of peak C (*m/e* 248), which was 14 mass units less than the tetramethyl Compound IV, was consistent with the trimethyldiamide III. Ions at *m/e* 176 $[M-(CH_3)_2NCO]^+$ and 72 $[(CH_3)_2NCO]^+$ indicated that peak C contained a dimethylamide moiety; the ion at *m/e* 58 $(CH_3NHCO)^+$ suggested that the parent molecule also contained a monomethylamide group. Further support for the presence of a monomethylamide function was derived from an intense ion at *m/e* 191, which can be accounted for by the loss of methylisocyanate by a McLafferty-type rearrangement of the molecular ion.

On-column methylation performed with deuterated I coupled with mass spectrometric analysis demonstrated that deuterium was incorporated into one methyl group in VI and into one and two methyl groups in III and IV, respectively. No deuterium was observed in VII under these conditions. However, deuterium was incorporated into the ester when the methylation was carried out in the presence of deuterated methanol[2], indicating that the source of the methyl group in this compound was the methanol solvent. When a mixture of I and II was allowed to stand for several days at room temperature and then neutralized with dilute hydrochloric acid, only the peaks corresponding to dimethylaniline and II were observed upon GLC analysis. This result indicates that the decomposition of the barbiturate in alkaline solution occurs after injection and not at ambient temperatures and that the decomposition is base catalyzed. Injection of the amides V and VI into the gas chromatograph with I in methanol did not result in the formation of the ester VII.

When II was injected into the gas chromatograph with 0.13 *M* tetramethylammonium hydroxide[3] in methanol, the results were more striking. As illustrated in Fig. 2*b*, II was almost completely degraded under these conditions to V–VII. Acidification of the reaction mixture prior to injection resulted in little or no decomposition of II upon injection.

DISCUSSION

A GLC–mass spectrometric study of the reaction of I and II shows that extensive degradation of the barbiturate occurs at high temperatures to form a number of products. This degradation is of interest, since phenobarbital is frequently determined by quantification of the area of its

[2] Stohler Isotope Chemicals.
[3] Supplied as 24% solution, Matheson, Coleman and Bell.

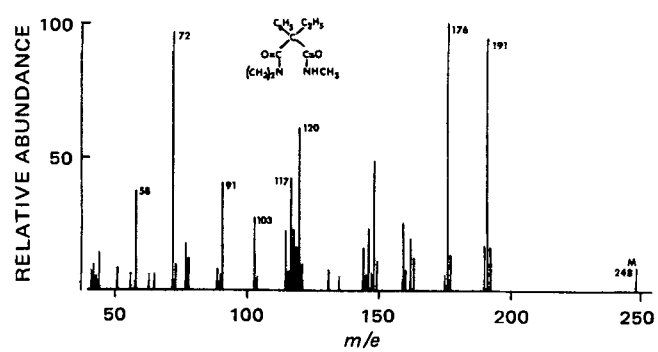

Figure 3—*Mass spectrum of peak C of Fig. 2.*

1,3-dimethyl derivative obtained after on-column methylation with I (1) and in at least one laboratory by quantification of the area of the early peak (7).

The degradation of II occurs in the injector port at high temperatures in the presence of base, since it does not occur in alkaline solutions at ambient temperatures or in neutral solutions at high temperatures. The first step in the degradation is likely to be the loss of a carbonyl group from II with the formation of *N,N*-dimethylphenylethylmalondiamide, which is then subsequently methylated to the tri- and tetramethyl derivatives. The formation of IV was observed previously in the reaction of phenobarbital with methylsulfinylmethide carbanion and methyl iodide (9) but not with phenobarbital and diazomethane (10). The methylated phenylbutyramides probably arise from III and IV, since it was shown that phenylethylmalondiamide forms V and VI when injected into a gas chromatograph with I (7), although this result was interpreted as either V and phenylethylmalondiamide having similar retention times (5) or the early peak being phenylethylmalondiamide (4).

The reaction pathway leading to the formation of VII has not been elucidated at this time, but it apparently is not formed from the methylated 2-phenylbutyramides. A possibility is that a 1,6-cleavage of II occurs, leading to a product that could form the ester with methanol. It was demonstrated that 1,6-cleavage in addition to 3,4-cleavage can occur in the degradation of barbiturates (11).

In view of the instability of II in solutions of I at high temperatures, the on-column methylation of phenobarbital might be expected to result in the formation of degradation products. The lack of the formation of significant amounts of products other than V indicates that reactions involving methylation and decomposition of I are completed before extensive degradation of II can occur. However, it does suggest that any analytical method for phenobarbital determination involving quantification of a decomposition product should be applied with caution, since the amounts of degradation products depend on experimental parameters such as injection port temperatures and the concentration of the methylating reagent.

EXPERIMENTAL

Materials—Compound II was prepared by reacting 2.3 g of phenobarbital[4] with 11.4 g of methyl iodide and 0.5 g of sodium hydroxide in 10 ml of methanol for 4 hr at ambient temperature. The product, which separated from the mixture upon dilution with water, was recrystallized from ethanol–water. The purified product, mp 88° [lit. (12) mp 84°],

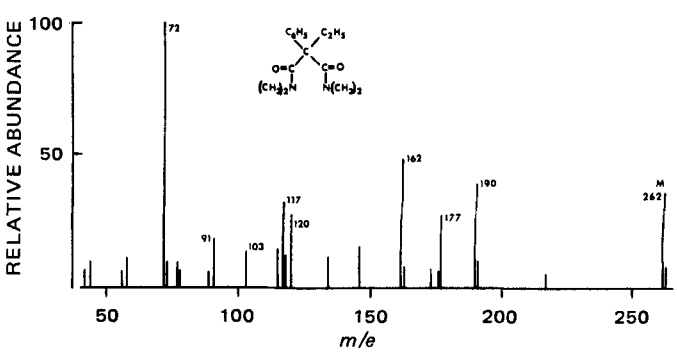

Figure 4—*Mass spectrum of peak D of Fig. 2.*

[4] Merck and Co.

chromatographed as a single peak with the same retention time as that observed by on-column methylation of phenobarbital; its mass spectrum was consistent with that previously reported (13). Compound I was prepared by the method of Brochmann-Hanssen and Oke (2), and deuterated I was prepared in a similar manner using methyl iodide-d_3[5]. A 1.8 *M* solution of I was obtained from a commercial source[6].

2-Phenylbutyryl chloride was prepared by refluxing 10 g of 2-phenylbutyric acid[7] and 15 g of thionyl chloride for 30 min and then distilling off the excess thionyl chloride. The acid chloride was divided into three portions. Compounds V and VI were prepared by reacting portions of the 2-phenylbutyryl chloride with 20 g of methylamide hydrochloride and 25 g of dimethylamine hydrochloride, respectively, which had been previously adjusted to about pH 12. The reaction mixture was allowed to stand overnight, during which time the crude products separated. The mixture was again made alkaline and extracted with ether. The extract was dried with anhydrous sodium sulfate, and the ether was evaporated. Compound V was obtained as a solid, which was purified by treatment with charcoal[8] in ethanol. Then the ethanol was evaporated, and the compound was recrystallized from hexane, mp 97° [lit. (14) mp 95–96°]. Compound VI was obtained as a viscous liquid, which was purified by repeated treatment with charcoal in ethanol.

Compound VII was prepared by refluxing 5 g of 2-phenylbutyric acid, 10 ml of methanol, and 0.2 ml of concentrated sulfuric acid for 4 hr. Then the cooled reaction mixture was poured into 25 ml of water and saturated with sodium bicarbonate. The alkaline solution was extracted with ether and dried overnight with anhydrous sodium sulfate, the ether was evaporated, and the ester was distilled, bp 225° [lit. (15) bp 228°].

Methods—GLC was performed on a 1.82-m × 3.2-mm o.d. glass column packed with 3% OV-17 on Chromosorb WHP[9]. The carrier gas was helium at a flow rate of 30 ml/min, the injector temperature was 250°, and the oven temperature was either maintained at 220° or programmed from 90 to 260° at 10°/min during the chromatography. A flame-ionization detector was used.

In the appropriate experiments, a portion of the effluent was passed through a heated glass jet separator into a mass spectrometer[10] operating in the electron-impact mode with an ionizing voltage of 70 ev. A mass range of 41–500 was automatically scanned at 2 sec/decade approximately every 5 sec during the elution of peaks. Normalized mass spectra of compounds included all ions within the mass range scanned with intensity greater than 5% of the base peak.

[5] Merck Sharp and Dohme Isotope Division.
[6] Supelco.
[7] Aldrich Chemical Co.
[8] Norit-A, Fisher Scientific Co.
[9] Applied Science Laboratories.
[10] DuPont 21-490 interfaced with a DuPont 21-094 data system.

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[x] To whom inquiries should be directed.

Use of Pharmacological Data for Bioavailability and Pharmacokinetic Analyses

PAUL A. KRAMER

Abstract □ The use of pharmacological responses such as pupil diameter for dosage individualization, bioavailability, and pharmacokinetic analyses is becoming more widespread. Attempts to use pupil diameter to assess morphine bioavailability illuminate the fact that multiple responses, nonlinearities, and the condition of the subject can produce misleading results unless the applicability of the method is confirmed.

Keyphrases □ Pharmacological response data—use in bioavailability and pharmacokinetic analyses, situations producing misleading results determined □ Bioavailability—analyses based on pharmacological response data, situations producing misleading results determined □ Pharmacokinetics—analyses based on pharmacological response data, situations producing misleading results determined □ Pupil diameter—measurements used to assess morphine bioavailability □ Morphine—bioavailability analyzed using pupil diameter measurements

Several recent publications underscored the utility of pharmacological response data for performing drug bioavailability and pharmacokinetic analyses (1–3). The theoretical foundation and limits of applicability of the approach were described in detail (2, 4), and this approach was applied successfully to several systems that would have been difficult or impossible to analyze by conventional means (5, 6). An obvious necessity for using pharmacological data is the existence of a clearly defined, measurable, graded response to the administered drug, but the availability and quantitation of such responses are often cited (7) as limiting factors in applying the method.

The objective of this study is to determine the usefulness of pharmacological methods for analyzing the bioavail-