Permethylation of Barbiturates: Variation in Product Ratios with Varying Methylsulfinylmethide Carbanion

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Abstract □ Permethylation of a barbiturate with the methylsulfinylmethide carbanion and methyl iodide gave three products clearly separable by GLC. Alteration of the reaction conditions by varying the barbiturate to carbanion ratio or the barbiturate to carbanion exposure time resulted in a single product for only one examined barbiturate, secobarbital. This derivatization technique is useful for the GLC analysis of polar barbiturate metabolites, such as glucuronides, in biological fluids but is of limited value for the analysis of the parent compounds.

Keyphrases □ Permethylation—barbiturates with methylsulfinylmethide carbanion and methyl iodide, GLC separation □ Barbiturates—permethylation with methylsulfinylmethide carbanion and methyl iodide, GLC separation □ GLC—determination, permethylation, barbiturate derivatives

A previous publication (1) noted that barbituric acid derivatives (I*a*–I*c*) were not stable under the conditions employed for the permethylation of peptides (2), sugars (3), and polar drug metabolites (4); the substrate in dimethyl sulfoxide was exposed to the methylsulfinylmethide carbanion (1 *M*) for a short period, after which methyl iodide or deuterated methyl iodide was added to complete the reaction. The reaction mixture contained three products, II, III, and IV, whose relative amounts were thought to be dependent only upon the nature of the alkyl groups at the 5-position of the parent compound (I) and upon *N*-substitution [*e.g.*, hexobarbital (I*a*) and mephobarbital]. Compounds II–IV were well-characterized by GLC–mass spectrometry for a series of six barbiturates, and their mass spectral fragmentation patterns were elucidated (1). Permethylation was shown not to be a practical technique for the quantitative analysis of barbiturates in biological extracts. However, it was considered a useful derivatization method for the GLC–mass spectrometry analysis of polar metabolites, especially glucuronides, in nonextracted biological fluids, such as rat bile, even though the structure of the barbiturate nucleus was altered (4).

The current study was an attempt to establish reaction conditions under which a single product could be formed. Two parameters were examined: the bar-

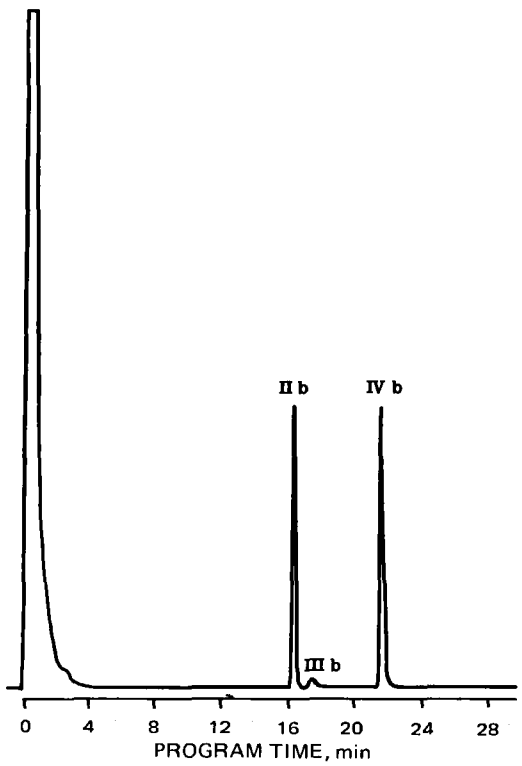


Figure 1—*GLC separation of a product mixture obtained from the permethylation of phenobarbital (I*b*). Reaction conditions were: 100 μg of phenobarbital (B), 5 μl of methylsulfinylmethide carbanion (C) (B/C = 0.09), 25 μl of methyl iodide, 15-min B to C exposure time, and 1-hour total reaction time. GLC conditions were as described in* Experimental *(using SE-30).*

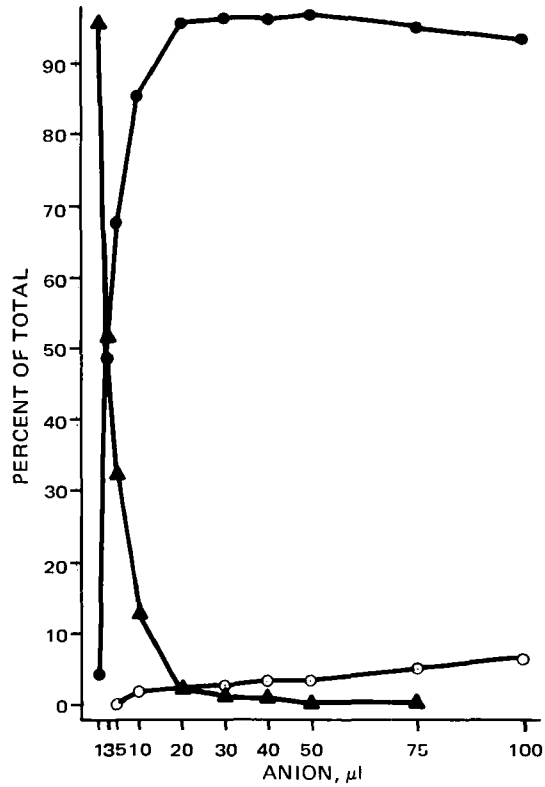
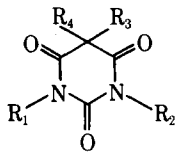


Figure 2—*Relative proportions of the products obtained from the permethylation of 100 μg of hexobarbital (I*a*) with varying amounts of methylsulfinylmethide carbanion. Key:* ▲, *II*a; ○, *III*a; *and* ●, *IV*a.

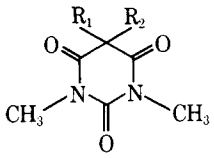


I

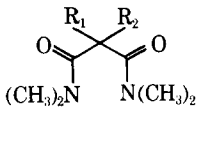
Ia: $R_1 = H$, $R_2 = CH_3$, $R_3 = CH_3$, R_4 = 1-cyclo-C_6H_9

Ib: $R_1 = H$, $R_2 = H$, $R_3 = C_2H_5$, $R_4 = C_6H_5$

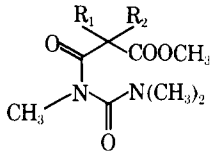
Ic: $R_1 = H$, $R_2 = H$, $R_3 = CH_2{=}CHCH_2$, $R_4 = CH_3(CH_2)_2CH(CH_3)$



II



III



IV

IIa, IIIa, IVa: $R_1 = CH_3$, R_2 = 1-cyclo-C_6H_9

IIb, IIIb, IVb: $R_1 = C_2H_5$, $R_2 = C_6H_5$

IIc, IIIc, IVc: $R_1 = CH_2{=}CHCH_2$, $R_2 = CH_3(CH_2)_2CH(CH_3)$

biturate to carbanion ratio and the length of barbiturate to carbanion exposure time prior to the addition of methyl iodide.

EXPERIMENTAL

Hexobarbital (Ia), phenobarbital (Ib), and secobarbital (Ic) analytical standards[1] were each dissolved in dimethyl sulfoxide[2] (2 μg/μl). Aliquots then were permethylated with the methylsulfinylmethide carbanion, prepared as previously described (2), and methyl iodide (always added in excess). The barbiturate to carbanion ratio was adjusted by varying either the barbiturate (micrograms) or the carbanion (microliters).

The exposure time prior to the addition of methyl iodide was usually 15 min, but it was the variable (0–30 min) in one experiment. The total reaction time was 1 hr. The reactions were stopped by the addition of water, the products were extracted into chloroform and washed three times with water, and the product mixture was obtained by evaporation of the chloroform under a gentle stream of nitrogen. The residue was redissolved in an amount of chloroform equal to the original amount of barbiturate for GLC and GLC–mass spectrometry analysis.

The separations were carried out using 1.8-m (6-ft) × 4-mm coiled columns[3] packed with 3% SE-30 on Gas Chrom Q (100–120 mesh) or 10% OV-17 on Gas Chrom Q (80–100 mesh)[4]. Operating parameters included: injector temperature, 250°; flame-ionization detector temperature, 300°; nitrogen flow rate, 30 ml/min; and temperature programming, from 125° at 4°/min. Quantification was by means of an automatic digital integrator[5].

All chromatographic peaks were identified with a combination gas chromatograph–mass spectrometer[6], with a 1.8-m (6-ft) × 4-mm coiled glass column packed with 1% SE-30 on Gas Chrom Q (100–120 mesh) as the inlet. The ionizing and accelerating potentials were 70 ev and 3.5 kv, respectively. The trap current was 60 μamp, the source temperature was 270°, the flash heater and separator temperatures were 250°, and the column oven temperature was programmed from 125° at 4°/min.

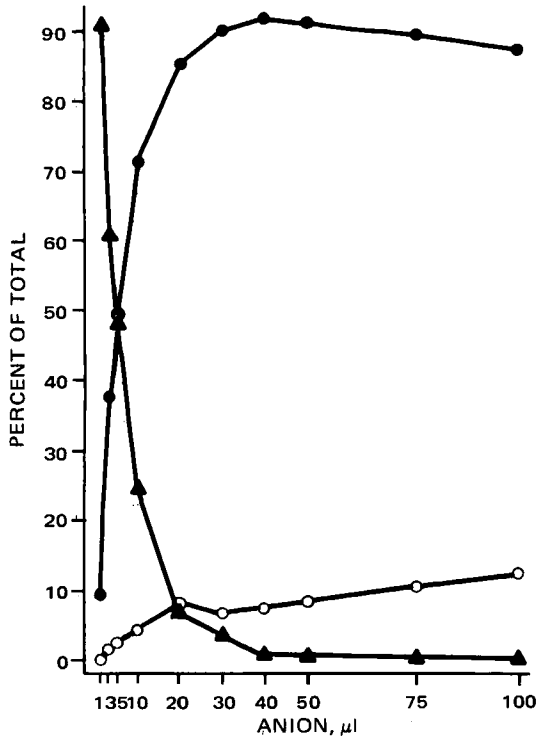


Figure 3—*Relative proportions of the products obtained from the permethylation of 100 μg of phenobarbital (Ib) with varying amounts of methylsulfinylmethide carbanion. Key:* ▲, *IIb;* ○, *IIIb; and* ●, *IVb.*

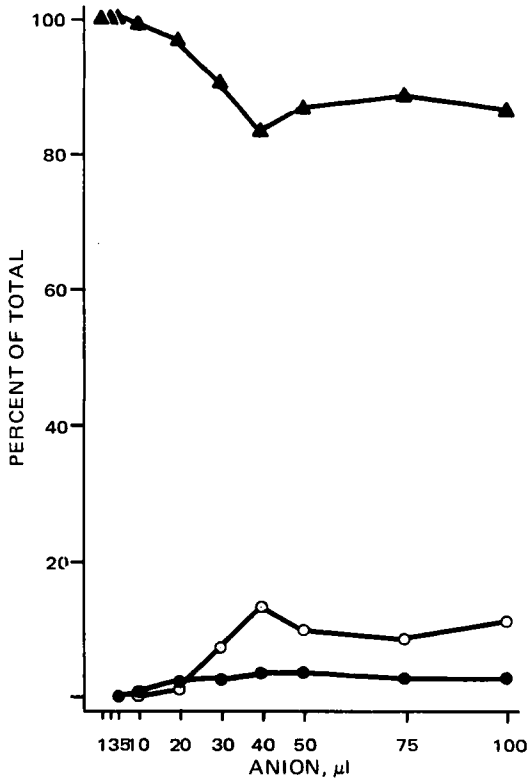


Figure 4—*Relative proportions of the products obtained from the permethylation of 100 μg of secobarbital (Ic) with varying amounts of methylsulfinylmethide carbanion. Key:* ▲, *IIc;* ○, *IIIc; and* ●, *IVc.*

RESULTS AND DISCUSSION

The three products (II, III, and IV) formed by permethylation of a barbiturate (I) were all clearly separated by GLC analysis on ei-

[1] Gifts from Mrs. Elizabeth Solow, Department of Neurological Surgery, Indiana University School of Medicine.

[2] Eastman Chemical Co., Spectro-grade; distilled over calcium hydride.

[3] Tracor model 550 dual-column gas chromatograph.

[4] Applied Science Laboratories, State College, Pa.

[5] Infotronics model CRS-204.

[6] LKB-9000-S.

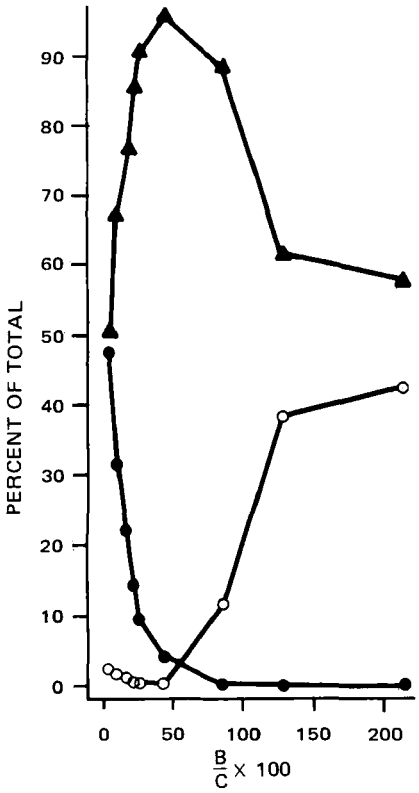


Figure 5—*Relative proportions of the three products obtained from the permethylation of phenobarbital (Ib) at varying barbiturate (B) to carbanion (C) ratios. Key:* ▲, *IIb;* ○, *IIIb; and* ●, *IVb.*

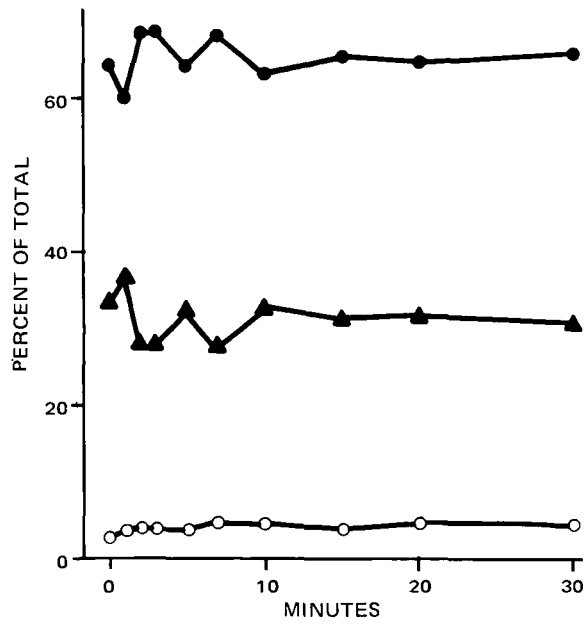


Figure 6—*Relative proportions of three products obtained from the permethylation of phenobarbital (Ib) at varying barbiturate to methylsulfinylmethide carbanion exposure times. Key:* ▲, *IIb;* ○, *IIIb; and* ●, *IVb.*

ther SE-30 or OV-17 columns (*e.g.*, *Ib* in Fig. 1). If the barbiturate to carbanion ratio was less than unity, the conversion to II–IV was complete. At a ratio >2, the total conversion to products was less than 50%. All detector responses were assumed to be essentially identical, because only relative yields are important to this discussion.

Figures 2–4 show the relative proportions of II, III, and IV obtained by varying the amount of methylsulfinylmethide carbanion used during the permethylation of a constant amount of *Ia* (0.45 μmole), *Ib* (0.43 μmole), and Ic (0.42 μmole), respectively. The lowest amount of carbanion used, 1 μmole, was a slight excess, assuming that the only product formed was the *N,N'*-dimethyl derivative, II. In two cases, *Ia* (Fig. 2) and *Ib* (Fig. 3), II (*a* or *b*) made up over 90% of the total product mixture when 1 μmole of carbanion was used, but this proportion rapidly decreased to zero above 50 μmoles (~60-fold excess). However, the relative proportion of IV (*a* or *b*) increased from <10% with 1 μmole of carbanion to >90% with 20–30 μmoles. The proportion of IV decreased slightly above 50 μmoles of carbanion with a concomitant rise in the proportion of III to between 5 and 10%.

The third case, Ic, was completely different from the previous two (Fig. 4). A quantitative yield of IIc was obtained with 1–5 μmoles of carbanion, and the relative proportion was never less than 83%. The relative proportion of IVc was always <5%. Compound IIIc leveled off between 8 and 13% at higher carbanion levels (≥40 μmoles). Therefore, Ic was the only barbiturate examined that formed a single derivative at any barbiturate to carbanion ratio. It is unlikely that any steric effect of the substituents at C-5 is the only factor governing the observation that Ic is much more stable to permethylation than either *Ia* or *Ib*, since all three barbiturates have bulky C-5 substituents. Electronic factors, such as the relative stabilities of the polyanions formed prior to the addition of methyl iodide, probably are more important.

An attempt was made to obtain a single product from *Ib* by using less than a 1:1 molar ratio of carbanion to barbiturate (Fig. 5). In Fig. 5, the values along the abscissa that are <50 reflect an excess of carbanion, again assuming that only the dimethyl derivative (*IIb*) forms. It is obvious that no single product could be formed, even when the barbiturate was in a greater than twofold excess. The relative proportion of IV*b* decreased to zero when the barbiturate to carbanion ratio was >0.85. After reaching a maximum of 96% at a ratio of 0.43, the proportion of II*b* also decreased surprisingly, accompanied by a very significant increase in III*b*. The total product yield began to decrease at barbiturate to carbanion ratios >1 and was finally <50% when this ratio was >2.

Another variable, which was considered to be of potential importance in studying the permethylation of barbiturates, was the length of time the barbiturate was exposed to the methylsulfinylmethide carbanion prior to the addition of methyl iodide. In an experiment in which exposure time was the variable (Fig. 6), there were slight fluctuations in the relative proportions of II*b*, III*b*, and IV*b* (from 0 to 10 min), but the overall result was that the proportions were constant enough to show that the barbiturate to carbanion exposure time is not a variable for this reaction.

In conclusion, methylating reagents such as diazomethane, trimethylanilinium hydroxide, or tetramethylammonium hydroxide are still to be preferred for derivatizing barbiturates prior to GLC and GLC–mass spectrometry analysis, although permethylation is most useful for polar metabolites such as glucuronides.

REFERENCES

(1) R. M. Thompson and D. M. Desiderio, *Org. Mass Spectrom.*, **7**, 989(1973).

(2) P. A. Leclercq and D. M. Desiderio, *Anal. Lett.*, **4**, 305(1971).

(3) H. Bjorndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem. Int. Ed. Engl.*, **9**, 610(1970).

(4) R. M. Thompson, N. Gerber, R. A. Seibert, and D. M. Desiderio, *Drug Metab. Dispos.*, **1**, 489(1973).

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