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Dimethylformamide Dimethylacetal as a Derivatizing Agent for GLC of Barbiturates and Related Compounds

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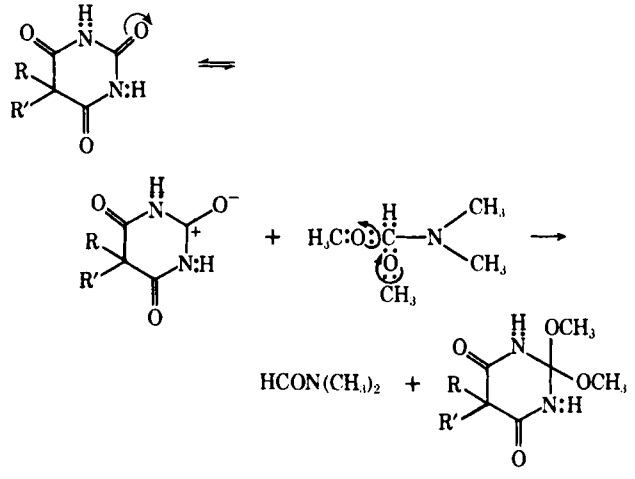
Abstract □ Dimethylformamide dimethylacetal reacted quantitatively and reproducibly with glutethimide, phenobarbital, hexobarbital, secobarbital, amobarbital, aprobarbital, and pentobarbital to form the corresponding acetals. The acetal derivative could be chromatographed easily on a 3% OV-17 column using either isothermal or programmed conditions (for multiple separations), permitting the separation and determination of the compounds studied with good precision and speed. NMR evidence and mass fragmentography was used to confirm the structure of the derivative. The glutethimide derivative was susceptible to solvent-induced reversibility.

Keyphrases □ Dimethylformamide dimethylacetal—used as derivatizing agent for GLC of barbiturates, glutethimide □ Barbiturates—GLC analysis, dimethylformamide dimethylacetal as derivatizing agent □ Glutethimide—GLC analysis, dimethylformamide dimethylacetal as derivatizing agent □ GLC—analysis, barbiturates and glutethimide, dimethylformamide dimethylacetal as derivatizing agent

The vast use of the 5,5-disubstituted barbituric acid derivatives, glutethimide, and methyprylon as sedatives as well as the interest they have commanded in forensic science has led to numerous reports on the use of GLC as an analytical method for their determination (1–5). The most recent literature (1–3) relied on the formation of their *N*-methyl derivatives prior to GLC (2) or on *in situ* formation in the chromatograph injector port (1–3). The derivative was formed by the action of trimethylanilinium hydroxide, a reagent requiring a time-consuming synthesis[1] (3). While the formation of the methylated compounds removed the disadvantages of adsorption, tailing, and column contamination previously experienced with GLC of the parent barbiturates, most of the columns and conditions used did not remove the failure of baseline separation when chromatographing mixtures.



Scheme I

The inadequacies prompted this laboratory to investigate other derivative routes, which led to a study of the chromatographic behavior of the product of barbiturates with dimethylformamide dimethylacetal (I)[2].

Depending on the reacting compounds and conditions employed, dimethylformamide–dialkylacetals react to form either acetals with imides (6) or formamidine derivatives with amides (7). Since I decomposes during the reaction to form both CH_3^+ and OCH_3^-, either *N*-methylation or acetal formation with barbiturates was seemingly possible. The path taken would depend only on the relative ease of proton abstraction from the subject compound, as opposed to carbonyl polarization.

[1] After completion of this work, Pierce Chemical Co. began marketing the reagent as MethElute.
[2] Aldrich Chemical Corp., Milwaukee, Wis.

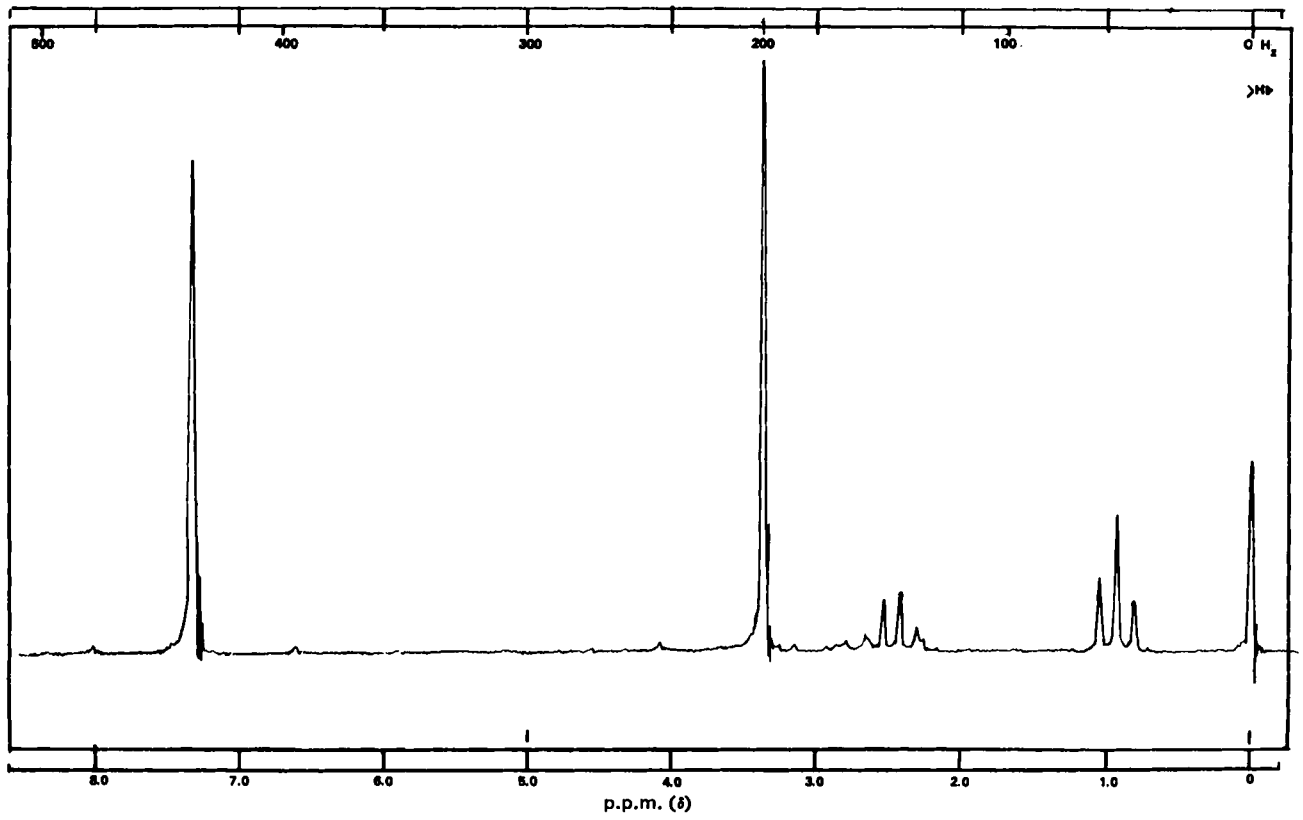


Figure 1—*NMR spectrum of phenobarbital dimethylformamide dimethylacetal adduct at 60 MHz. (concentration 45 mg./0.5 ml. $CDCl_3$; spectrum amplitude 2.0; 40 r.p.s. spin; 250 sec./500 Hz. sweep rate; filter 1; R_f level 0.08).*

Table I—Retention Time and Response Ratio Data

Compound[a]	Relative Retention Time [Dibutyl Phthalate]	Relative Retention Time [*n*-Eicosane]	Response Ratio (Triplicate Average) Dibutyl Phthalate	Response Ratio (Triplicate Average) *n*-Eicosane	Concentration, mg./ml.
Aprobarbital	0.413	0.565	0.7938	0.5442	7.96
Amobarbital	0.481	0.661	0.8529	0.5819	8.15
Pentobarbital	0.519	0.714	0.8516	0.5712	8.05
Secobarbital	0.572	0.786	0.8334	0.5806	6.99
Hexobarbital	0.865	1.19	0.7962	0.5499	4.81
Phenobarbital	0.923	1.27	0.9197	0.6270	8.84
Methyprylon	0.542	—	0.8599[b] (0.8702)[c]	—	4.52[b]
Glutethimide	—	1.29[d,e]	—	0.2749	12.08
Dibutyl phthalate	1.00 (10.4 min.)	1.38	—	—	—
n-Eicosane	0.754 (7.49 min.)	1.00[e]	—	—	—

[a] As the acetal (Compounds 1–6). [b] Direct solution in dimethylformamide. [c] Solution in 0.5 ml. AcOH in dimethylformamide (5 ml. total volume). [d] As the acetal; no solvent; *n*-eicosane standard. [e] At 240° isothermal, retention times of *n*-eicosane = approximately 1.9 min. and of glutethimide acetal = 3.1 min.

EXPERIMENTAL[3]

Instrument Parameters—The gas chromatograph for the analytical studies was equipped with dual U-shaped Pyrex columns, 1.8 m. × 0.6 cm. (6 ft. × 0.25 in.) × 4 mm. i.d. ("on-column" inserted), packed with 3% OV-17 on Supelcoport (100–120 mesh)[4]. Sensitivity was 10^{-10} amp./mv., with helium flow at 55 ml./min., hydrogen flow at 40 ml./min., and air at 1.6 ft.³/hr. The injector temperature was 285°, the detector temperature was 300°, and the column was programmed from 160 to 220° at 7°/min.

GC–mass spectrometry conditions were: glass coil column, 0.6 cm. × 1.8 m. (0.25 in. × 6 ft.) × 2.8 mm. i.d.; injector temperature, 210°; separator temperature, 210°; column, 160–200° at 8°/min.; flow, 25 ml./min.; high vacuum, 8 × 10^{-6} torr; transfer line, 200°, manifold, 120°; beam current, 0.30 ma.; ion energy, 9.2 v.; electron energy, 70.3 v.; collector, 34.2 v., emission, 0.70 ma.; extractor, 10.2 v.; lens, 100.9 v.; and high voltage, 1.55.

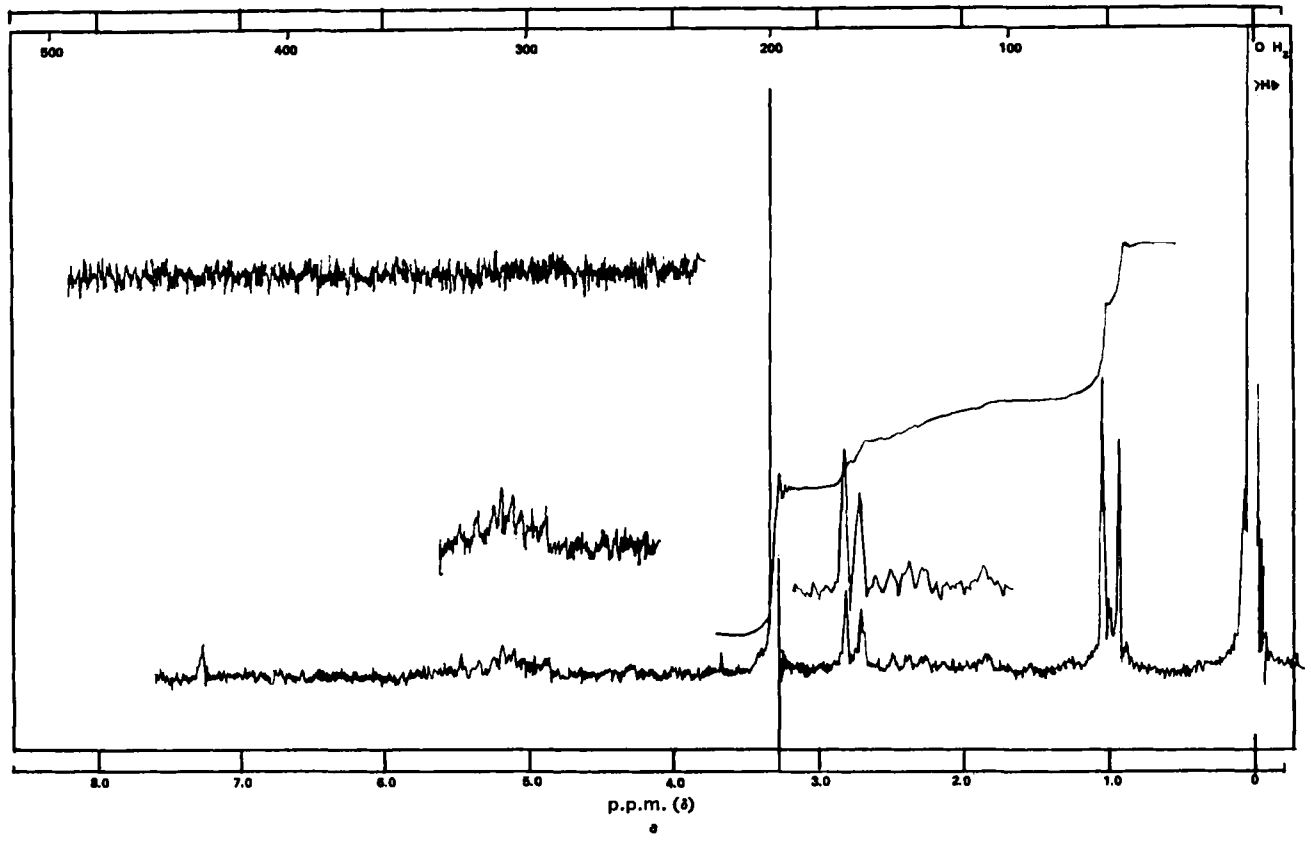
Standard Procedure—Accurately weigh 5–40 mg. of the subject compound[5] into a 5-ml. volumetric flask, add exactly 1.0 ml.[6] of I, swirl gently until the compound dissolves, and warm on a steam bath for 10 min. Cool, dilute with internal standard solution, and

[3] GLC analyses were performed using a Bendix model 2500 chromatograph equipped with a flame-ionization detector, Honeywell Electronik 194 recorder, and a Disc integrator. All area measurements were taken from the integrator with the aid of a model 610 Disc printer. Mass fragmentography was performed on a Varian 1400 chromatograph linked to a Finnigan Corp. 3000/PDP 8-E system. NMR analyses were performed on a Varian T-60 spectrometer.

[4] Supelco Corp.

[5] Reference standards were obtained from the respective manufacturers except phenobarbital (Merck) and pentobarbital (Ganes Chemical Co.).

[6] For small amounts of compound (5–10 mg.), 0.5 ml. of reactant will suffice.



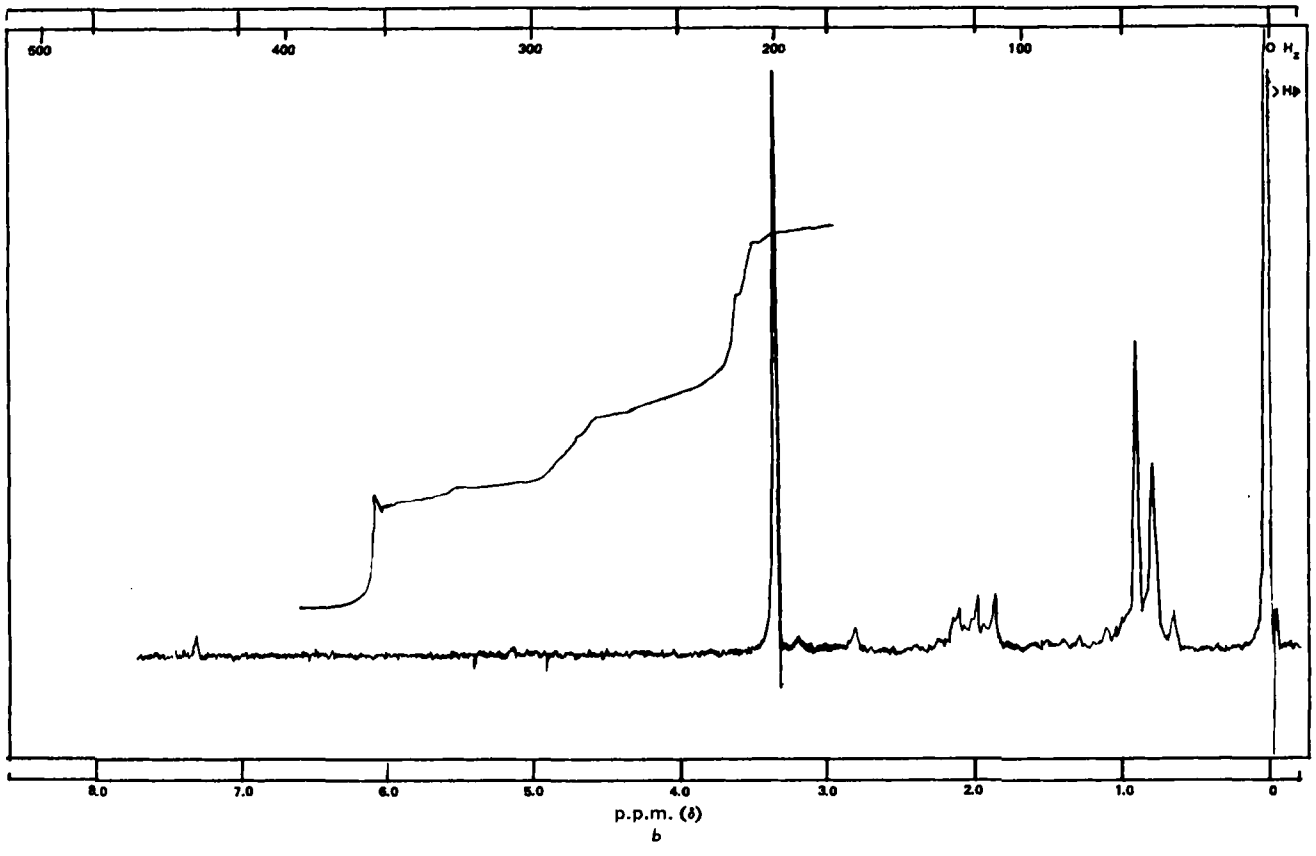


Figure 2—(a) *NMR spectrum of aprobarbital dimethylformamide dimethylacetal adduct at 60 MHz. (concentration 23 mg./0.4 ml. $CDCl_3$; amplitude 20; 52 r.p.s.; 250 sec./500 Hz. sweep rate; filter 2; R_f level 0.11; upper trace amplitude 40; insert sweep offset 280 Hz., amplitude 50).* (b) *NMR spectrum of amobarbital dimethylformamide dimethylacetal adduct at 60 MHz. (concentration 44 mg./0.5 ml. $CDCl_3$; amplitude 12.5; 58 r.p.s.; 250 sec./500 Hz. sweep rate; filter 2; R_f level 0.065).*

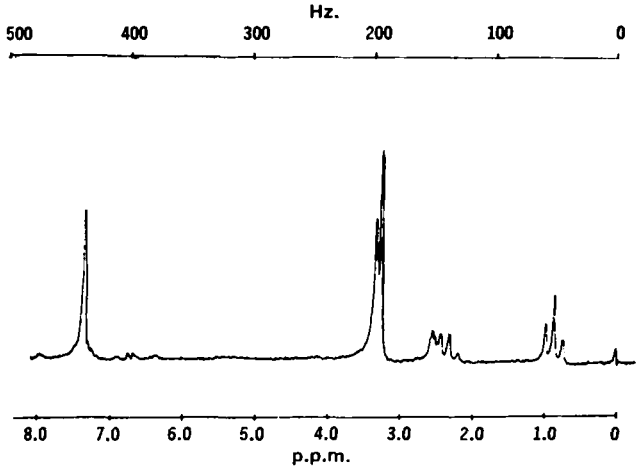


Figure 3—*NMR spectrum of metharbital dimethylformamide dimethylacetal adduct at 60 MHz. (concentration 46 mg./0.6 ml. dimethyl sulfoxide-d_6; amplitude 12.5; 48 r.p.s.; 250 sec./500 Hz. sweep rate; filter 2; R_f level 0.09; parts per million on σ scale). Note dimethyl sulfoxide-d_6 signal at 152 Hz. overlaps —CH_2 quartet of —C_2H_5 group at 142 Hz.*

Methyprylon Procedure—Since methyprylon reacts with the reagent only with difficulty, it is simply dissolved in dimethylformamide or chloroform and chromatographed directly in the concentrations specified for the other sedatives.

Mixtures of Sedatives—Samples containing mixtures in the 5–40-mg. range can be prepared in identical fashion. If the combined total in the mixture is in excess of about 100 mg., use an additional 0.5 ml. of reactant for each additional 50 mg.

RESULTS AND DISCUSSION

Dimethylformamide reacted quantitatively with the sedatives studied (except methyprylon) in such a manner that simple —N—H to N—CH_3 transformation was not considered a possibility. Since the compounds studied are chemically related to the imides studied by Meerwein *et al.* (6), the mechanism shown in Scheme I was favored. This mechanism takes advantage of both the CH_3^+ and the OCH_3^- formed by the acetal. A derivative of this nature is somewhat unusual but would depend only upon ease of polarization of the carbonyl on C-2. Since this position is known to polarize easily (8) and is less hindered, it was considered the only logical position of reaction. While the actual derivatives formed showed GLC retention times similar to the 1,3-dimethyl derivatives noted previously (1–3), simple 1,3-dimethyl formation was excluded because of ease of formation and because it was noted that the barbiturate must be present as the keto tautomer for smooth reaction to occur. Thus,

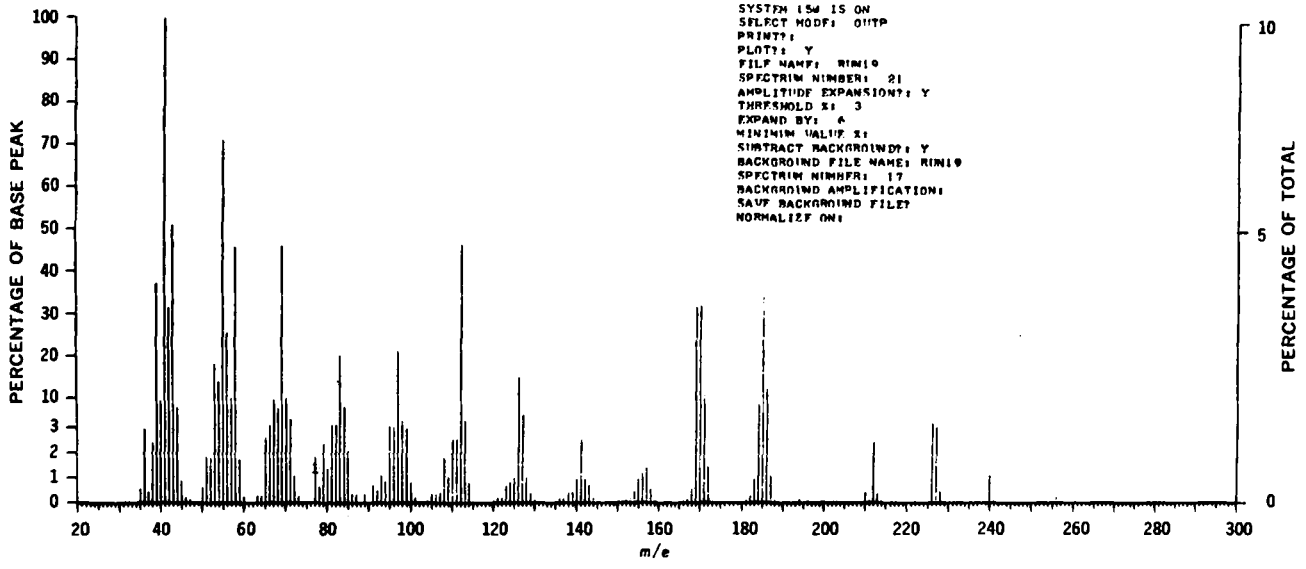


Figure 4—*Mass fragmentogram of amobarbital dimethylformamide dimethylacetal adduct from 1-μl. injection of 8.25 mg. of amobarbital, 1 ml. of reagent/5 ml. volume in dimethylformamide.*

then dilute to the mark with dimethylformamide[7]. For the internal standard, weigh accurately about 4 g. of dibutyl phthalate[8] into a 100-ml. volumetric flask, mix, and dilute to volume with reagent grade chloroform. Use 1.0 ml. of internal standard for approximately 20 mg. of barbiturate.

The sample solution with standard was injected (3 μl.) into the chromatograph using the prescribed operating conditions. In place of dibutyl phthalate, 1 ml. of a chloroform solution of *n*-eicosane/20 mg. of compound (concentration 12 mg./ml.) may be used.

Record the peaks and areas using a recorder equipped with an integrator (Disc) or digital integrator and calculate the response ratio and concentration of compounds of unknown purity using the appropriate standard equations.

Glutethimide Procedure—The standard procedure is changed by the addition of about 12 mg. (accurately weighed) of *n*-eicosane to the I-glutethimide mixture after heating. After the *n*-eicosane dissolves, 1 μl. is injected into the chromatograph, using the prescribed conditions, without further solvent dilution.

[7] Baker Instra Analyzed.
[8] Fisher reagent grade.

it was noted that the sodium salts of secobarbital and amobarbital reacted in a nonpredictable fashion, resulting in multiple chromatographic peaks. However, both reacted smoothly as the free acids alone or in a mixture.

Proof of acetal formation rather than methylation was obtained by the use of NMR and mass fragmentography. NMR analysis in $CDCl_3$ of the isolated adduct from the reaction of phenobarbital (Fig. 1), aprobarbital (Fig. 2*a*), and amobarbital (Fig. 2*b*) as well as metharbital in dimethyl sulfoxide-d_6 (Fig. 3) clearly indicated derivative formation of the methoxy type. Figure 1 shows singlet resonance at 203 Hz. of relative area 6, 2*a* shows a singlet at 199 Hz. (area 6) and N—H at 308 Hz., and 2*b* shows a singlet at 201 Hz. (area 6). In support of these findings, the isolated product of the reaction of metharbital gave an NMR spectrum which showed two nonequivalent —CH_3 patterns, the resonance at 199 Hz. being attributed to the N—CH_3 and that at 195 Hz. resulting from >$C(OCH_3)_2$. The former matches that shown in the spectrum of unreacted metharbital, while the peak at 195 Hz. (6 area units compared with the phenyl area of 5) could only indicate acetal formation at a carbonyl because of the existence of CH_3 on position 1 (area 3) and the absence of any other possible nonequivalent CH_3 resonance in the vicinity of 195 Hz.

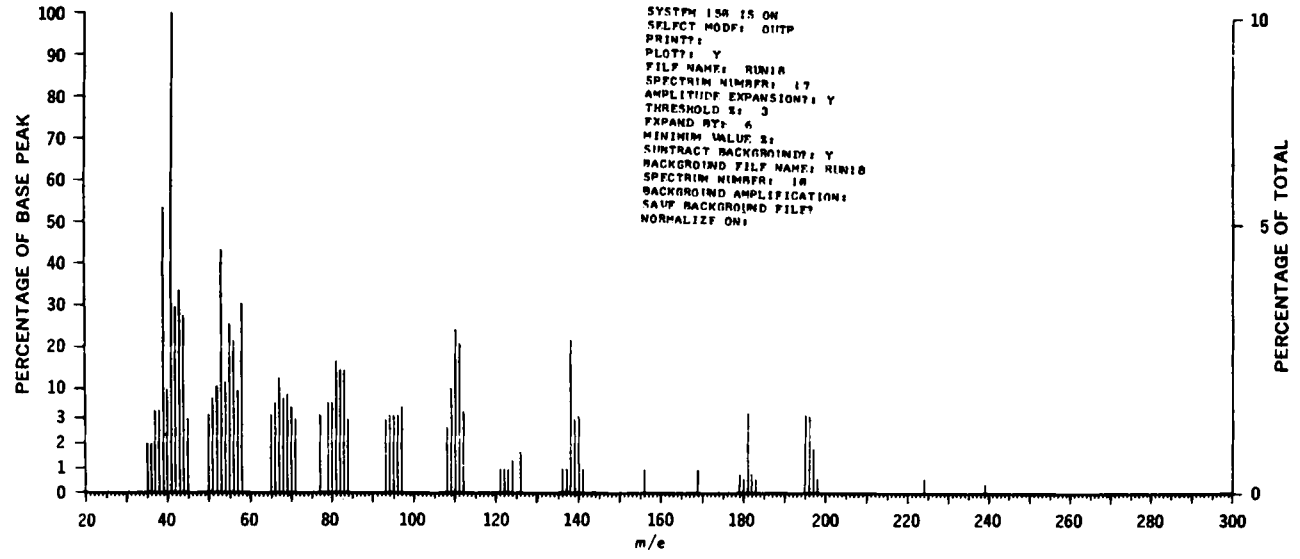


Figure 5—*Mass fragmentogram of aprobarbital dimethylformamide dimethylacetal adduct from 1-μl. injection of 8.77 mg. aprobarbital, 1 ml. of reagent/5 ml. volume in dimethylformamide.*

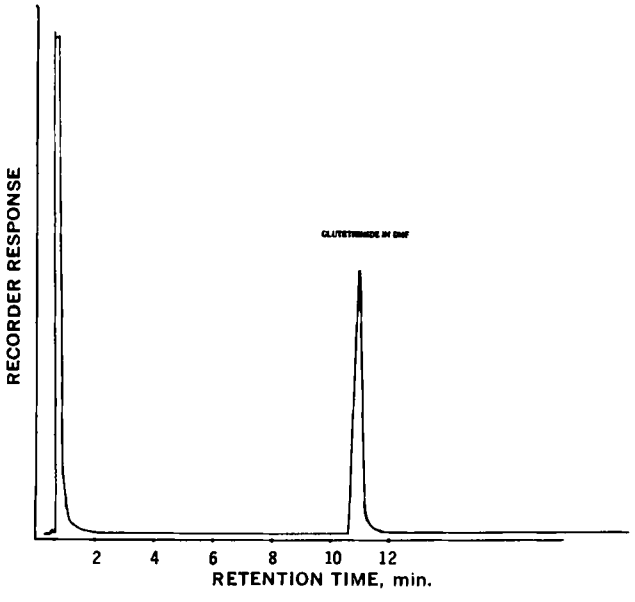
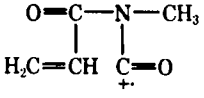


Figure 6—*Gas–liquid chromatogram of glutethimide (12.12 mg./ml.) in dimethylformamide; programmed conditions. Retention time = 11.05 min.*

Mass fragmentography of the reaction mixtures of amobarbital and aprobarbital, using the conditions previously given, was carried out as further proof of derivative formation. The resulting data were compared directly with those published by Knight (2). The patterns for amobarbital acetal (A) (Fig. 4) and aprobarbital acetal (B) (Fig. 5) are dissimilar to those given for the corresponding 1,3-dimethyl barbiturates (2). A partial analysis of the fragmentations typically showed what might be expected for acetals (9, 10). Distinguishing features in the pattern of A are *m/e* 257 ($M^+ - 15$, $-CH_3$), *m/e* 226 ($M^+ - 46$; $-CH_3$, $-OCH_3$), and *m/e* 195 ($M^+ - 77$; $-2-OCH_3$, $-CH_3$, $-H$). A distinguishing feature in the pattern of B was the absence of a significant *m/e* 91 peak, which showed 35% of base peak abundance during the fragmentation of 1,3-dimethylaprobarbital (2). This may be attributed to the loss of CH_3 from:



which is possible to a significant extent only in the dimethyl compound having an unsubstituted carbonyl at position 2. Other mass fragments suggestive of acetal presence in B were *m/e* 224 ($M^+ - OCH_3$, $-H$) and *m/e* 181 [$M^+ - OCH_3$, $-H$, $-CH(CH_3)_2$], along with $M^+ - 73$, $M^+ - 87$, and $M^+ - 101$, typical of the fragmentation of acetals (10).

The proposed mechanism explains, in part, the reason why methyprylon formed the acetal derivative with extreme difficulty and why the glutethimide derivative was susceptible to solvent-induced reversibility. In the case of methyprylon, both the 2- and the 4-carbonyl position are sufficiently hindered by the 3,3-diethyl substituent. In addition, the 4-position does not have imide character and, therefore, should not derivatize in this manner. With glutethimide, the imide carbonyl at C-6 is open to acetal formation but would not be as stable as the $-NHC(OCH_3)_2NH$ formed with the barbiturates. The reversibility of the glutethimide reaction can be noted by a comparison of Figs. 6 and 7.

Linearity of detector response was established for the six barbiturates examined (concentration range 2–30 mg./ml.). Secobar-

Table II—Recovery Data: Known Standards

Compound	Replicate Analyses	Concentration, mg./ml.	Percent Recovery[a]	σ	Coefficient of Variation, %
Hexobarbital	6	10.02	100.4	±0.4412	0.439
Phenobarbital	7	10.14	98.54	±0.4702	0.477
Amobarbital	6	9.93	98.93	±0.7712	0.780
Aprobarbital	6	9.74	98.50	±0.5899	0.599
Pentobarbital	6	9.43	99.13	±0.7033	0.709
Secobarbital	6	9.97	99.48	±0.5270	0.530
Methyprylon[b]	6	4.52	99.40	±0.5254	0.529

[a] Values are based upon replicates on a single preparation. [b] Direct injection of solution made with 0.5 ml. AcOH in dimethylformamide (5 ml. total volume).

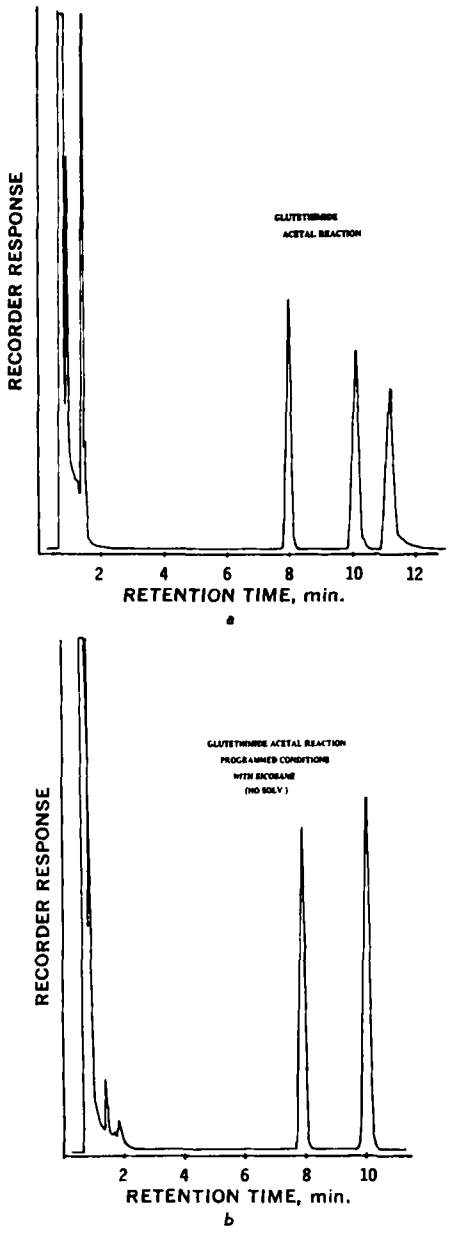


Figure 7—(a) *Gas-liquid chromatogram of glutethimide dimethylformamide dimethylacetal product with dibutyl phthalate internal standard (solvent mixture; $CHCl_3$; dimethylformamide acetal). Peak 1 = glutethimide acetal (8.03 min.); 2 = dibutyl phthalate (10.15 min.); and 3 = free glutethimide (11.2 min.). Chromatogram taken 60 min. after reaction.* (b) *Gas-liquid chromatogram of glutethimide dimethylformamide dimethylacetal product with* n-*eicosane (peak 1, 7.84 min.) without added solvent under programmed conditions. Chromatogram taken 60 min. after reaction.*

bital and amobarbital linearity showed coincident slopes. The retention time and response ratio data are presented in Table I. The relative retention times are, in increasing order, aprobarbital through phenobarbital, as expected by virtue of the effect of the substituents at the 5-position on the overall polarity and adsorbability of the molecule. No generalizations can be made for methyprylon and glutethimide with regard to elution characteristics since they do not follow the general barbiturate analyses.

An inspection of Table I as well as Figs. 8 and 9 clearly shows that, under programmed conditions, most combinations of barbiturates and methyprylon or glutethimide could be determined with minor procedural modifications. It is possible that methyprylon would interfere with baseline separation of pentobarbital and/or secobarbital (dibutyl phthalate standard) or glutethimide with phenobarbital (*n*-eicosane standard), although the probable need for such a separation would be remote.

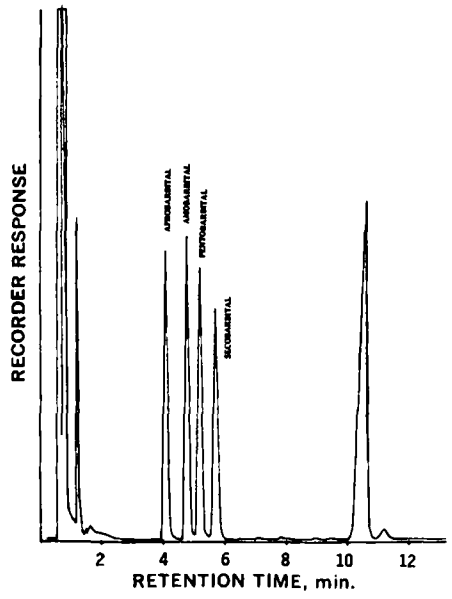


Figure 8—*Chromatogram showing the separation of the four noted barbiturates in a single mixture. Last major peak = dibutyl phthalate (minor peak is impurity in dibutyl phthalate).*

To evaluate the precision of the method and the quantitative recovery that can be anticipated, a series of standard barbiturates were analyzed (Table II). The recoveries obtained ranged from 98.54 to 100.4% based upon response to dibutyl phthalate internal standard. The standard deviation range was from ±0.4412 to ±0.7712.

Since one facet of the proposed method is the ease of determination of mixtures, two standard mixtures of bulk drugs were tested. Table III shows the results of determinations of: (*a*) pentobarbital plus hexobarbital, and (*b*) phenobarbital plus hexobarbital (Fig. 10) under programmed conditions and also at 215° isothermal. In addition, a commercial sample of sodium amobarbital with secobarbital sodium capsules[9] and a sodium pentobarbital capsule were

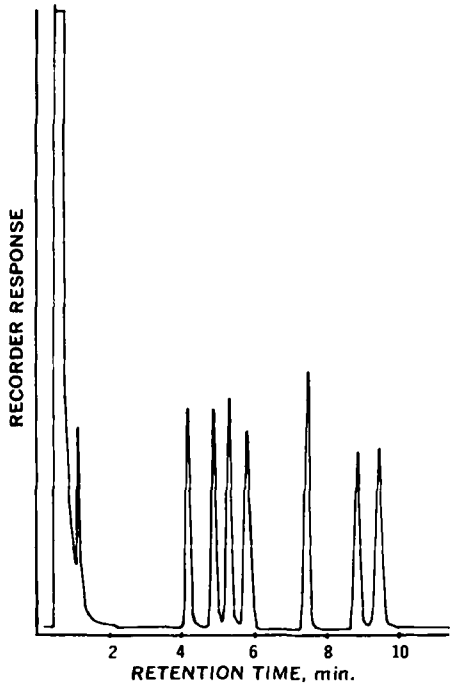


Figure 9—*Chromatogram showing the separation of aprobarbital, amobarbital, pentobarbital, secobarbital,* n-*eicosane, hexobarbital, and phenobarbital in their order of elution. The barbitals are as their acetals.*

[9] Tuinal.

Table III—Recovery Data: Barbiturate Mixtures

Mixture	Amount Used, mg.	Amount Found, mg.[a]	Percent Recovery[b]	Percent Theoretical
Phenobarbital–hexobarbital[c]	24.18 22.00	24.34 22.04	52.47 47.71	52.37 47.63
Phenobarbital–hexobarbital[d]	24.48 20.44	24.53 20.40	54.61 45.42	54.50 45.50
Phenobarbital–hexobarbital	19.93 21.85	20.05 22.05	47.98 52.77	47.70 52.30

[a] Average of duplicate determinations. [b] Relative to the mixture. [c] Programmed conditions. [d] Isothermal conditions (215° column temperature. Retention times: hexobarbital, 5.0 min.; phenobarbital, 5.82 min.; and dibutyl phthalate, 7.01 min.).

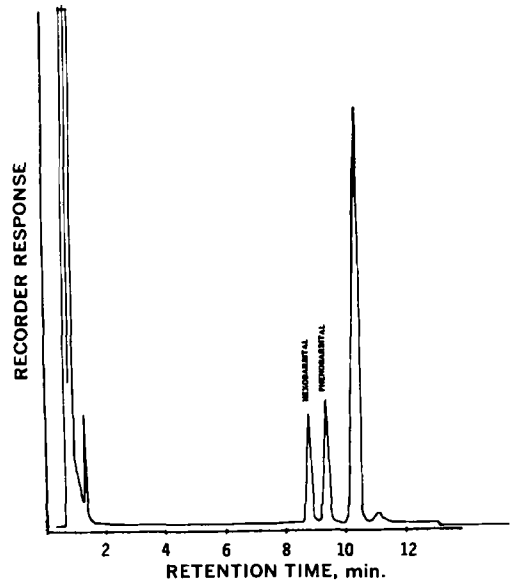


Figure 10—*Chromatogram of a mixture (Table III) of hexobarbital and phenobarbital with dibutyl phthalate internal standard, using the standard programmed conditions.*

assayed. The former assayed at 95.0% of the labeled amount (200 mg./capsule) when determined on two separate samplings. In the latter case, a single capsule assayed at 92.3% of the labeled amount (assuming 100 mg./capsule). The USP XVIII extraction procedure (11) for the respective dosage form was used, followed by evaporation of an aliquot of the chloroform extract to dryness (25°, stream of nitrogen) prior to acetal reaction.

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