Identification and Synthesis of *O*-Methylcatechol Metabolites of Phenobarbital and Some *N*-Alkyl Derivatives

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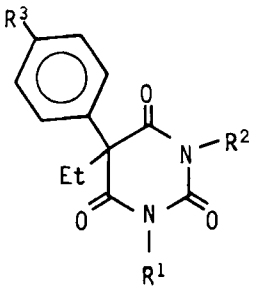
Abstract □ 5-Ethyl-5-(4-hydroxy-3-methoxyphenyl) barbituric acid was identified as a new, minor metabolite of phenobarbital in man. The identity of this *O*-methylcatechol metabolite was confirmed by an unequivocal chemical synthesis, and by GC–MS studies. Mephobarbital and the 1,3-dimethyl, 1-ethyl, and 1,3-diethyl analogues of phenobarbital yielded the corresponding *N*-alkylated *O*-methylcatechol metabolites, all of which were confirmed by synthesis. The *N*-alkyl barbiturates each gave additionally at least one *O*-methylcatechol metabolite in which *N*-dealkylation had occurred. These metabolites accounted for ~1–5% of the orally administered dose in man.

The metabolism of the anticonvulsant barbiturates phenobarbital (**1a**) and mephobarbital (**1b**) in animals and humans has been extensively studied and well reviewed.[1,2] Both phenobarbital[3] and mephobarbital[4] undergo oxidation in the 5-phenyl substituent to yield the corresponding *p*-hydroxy derivatives (**1c** and **1d**, respectively) as major metabolites; 3,4-dihydrodiols and 3,4-diols have also been reported.[5] Hooper et al.[6] presumptively identified from mass spectral data *O*-methylcatechol metabolites of phenobarbital and mephobarbital (**11a** and **11b**, respectively, Scheme I) in the urine of volunteers taking mephobarbital. We have now synthesized these 4-hydroxy-3-methoxy derivatives of both phenobarbital and mephobarbital and shown them to be identical to the metabolites found in humans. The synthetic route (Scheme I) involved preparing from the commercially available compound eugenol (**2**) an appropriately substituted malonic ester derivative in which the 4-hydroxy group was protected as a benzyl ether (**8**). This intermediate was condensed with urea, yielding the protected barbiturate (**9**), from which the benzyl group was removed by hydrogenation directly, or following *N*-alkylation.

We have recently reported the synthesis of the *N*-methyl and *N*-ethyl derivatives of phenobarbital for use as substrates in studies of metabolic structure–activity relationships.[7] Three of these derivatives (1,5-diethyl-5-phenylbarbituric acid, ethylphenobarbital, **1e**; 1,3-dimethyl-5-ethyl-5-phenylbarbituric acid, dimethylphenobarbital, **1f**; and 1,3,5-triethyl-5-phenylbarbituric acid, diethylphenobarbital, **1g**), also gave the corresponding *O*-methylcatechol metabolites (**11c**, **11d**, and **11e**, respectively), whose syntheses are reported here (Scheme I).



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	R^1	R^2	R^3
a	H	H	H
b	Me	H	H
c	H	H	OH
d	Me	H	OH
e	Et	H	H
f	Me	Me	H
g	Et	Et	H

Experimental Section

Materials—Phenobarbital (B.P. grade) was purchased from Queensland Ethicals, Brisbane, Qld. Mephobarbital (B.P. grade) was supplied by Sterling Pharmaceuticals, Sydney, N.S.W. Dimethylphenobarbital, ethylphenobarbital, and diethylphenobarbital were synthesized[7] and recrystallized to analytical purity, which was confirmed by GC and GC–MS. β-Glucuronidase (EC 3.2.1.31; Sigma Type H-2 from *Helix pomatia*), and *N,N*-dimethylacetamide were purchased from Sigma Chemical Co. (Saint Louis, MO). Tetramethylammonium hydroxide (24 g/100 mL of methanol) was purchased from Pfaltz and Bauer, Stamford, CT. 1-Iodopropane was purchased from Aldrich Chemical Co., Milwaukee, WI. All other reagents were of analytical grade purity.

Metabolic Studies in Humans—Single oral doses (300 mg) of each barbiturate, phenobarbital, mephobarbital, dimethylphenobarbital, ethylphenobarbital, and diethylphenobarbital, were administered orally to healthy adult male volunteers at intervals of not less than 6 weeks. Following each dose, all urine was collected daily for at least 10 d (or more frequently during Day 1). Urine volumes were recorded, and an aliquot was stored at −20 °C until analyzed. Urine specimens were investigated by gas chromatography–chemical ionization MS to identify drug metabolites. Aliquots of urine (1 mL) were incubated at 37 °C for 16 h with β-glucuronidase (1000 Fishman units) at pH 5 in acetate buffer. Following hydrolysis of conjugates, the sample was extracted with three 5-mL portions of ethyl acetate, and the combined extracts were evaporated under a nitrogen stream. The residue was taken up with *N,N*-dimethylacetamide (40 μL), then treated in turn with tetramethylammonium hydroxide (5 μL) and 1-iodopropane (10 μL). After 10 min of standing and brief centrifugation, 1–5-μL aliquots were injected into the GC/MS (Finnigan 3300F coupled to a Finnigan 6110 data system). The instrument was equipped with a 1.5 m × 2 mm id glass column packed with 3% OV-101. The carrier gas, methane (18 mL/min), also served as the reagent gas for the mass spectrometer, which was operated in the chemical ionization (CI) mode (positive ions). The source pressure was 1 torr and the source temperature was 135 °C. Spectra were acquired repetitively at intervals of 4 s over the scan range 100–500 amu, while the oven temperature was programmed to change from 130 to 280 °C at a rate of 6°/min.

Reference compounds were dissolved in methanol (0.1 mg/mL), and an appropriate quantity (1–5 μg) was dispensed into a tapered tube. The methanol was removed in an air stream, the residue was derivatized in exactly the same manner as the urine extract, and 1 μL was applied to the GC–MS.

The availability of reference standards permitted quantification of the *O*-methylcatechols in all urine samples by GC–MS.

Synthesis of the *O*-Methylcatechol Reference Compounds—Melting points were measured in an electrically heated silicone oil bath and are uncorrected. The NMR spectra were recorded on either a Varian EM-360 or a Jeol MH-100 spectrometer with tetramethylsilane or 3-trimethylsilanepropanesulfonate as the internal standard. Electron-impact mass spectra were taken on an A.E.I. MS-902 mass

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spectrometer. Microanalyses were performed by the Microanalytical Service of the University of Queensland.

Synthetic samples of the metabolites were prepared as outlined in Scheme I. The starting material, eugenol [2-methoxy-4-(2-propenyl)-phenol (2)], was obtained from the Sigma Chemical Co. (St. Louis, MO).

Carbethoxyeugenol (3)—A solution of sodium hydroxide (6.4 g, 160 mmol) in water (20 mL) was added slowly, with cooling and stirring, to a mixture of eugenol (25 g, 152 mmol) and ethyl chloroformate (17.4 g, 160 mmol). The reaction mixture was extracted with ether (3 × 50 mL), and the combined extracts were washed with water, dried (anhydrous $MgSO_4$), and evaporated to an oil. Vacuum distillation (bp 135–139 °C at 1.5 mm Hg) gave the derivative **3** as a colorless oil (31 g; 81%) which solidified on cooling (mp ~30 °C) as reported previously.[8]

4-Carbethoxy-3-methoxyphenylethanoic acid (4)—Compound **3** (23.0 g, 97 mmol) was dissolved in a mixture of glacial acetic acid (150 mL) and ethyl acetate (40 mL), and the resultant solution was ozonized at 0 °C until excess ozone was detected (potassium iodide–boric acid). Hot water (100 mL) was added to the reaction mixture which on cooling deposited **4** as a white crystalline solid (18.0 g, 73%), mp 124–125 °C (Lit.[8] 125–126 °C); MS: $M^{+\cdot}$ 254.

Methyl 4-carbethoxy-3-methoxyphenylethanoate (5)—An ethereal solution of diazomethane (30 mL of a 0.5 M solution, 15.0 mmol) was added in a dropwise manner to **4** (2.0 g, 8.4 mmol) in 20 mL of dry ether until a persistent yellow color prevailed. The solution was then stirred for 30 min at room temperature, the solvent was removed under reduced pressure, and the yellow residue oil was distilled under reduced pressure. The fraction bp 155–156 °C at 1.0 mm Hg solidified to give **5** as colorless crystals (1.8 g, 85%), mp 51–53 °C; MS: $M^{+\cdot}$ 268; ^1H NMR ($CDCl_3$): δ 2.31 (t, 3, CH_2CH_3), 3.60 (s, 2, CH_2), 3.71 (s, 3, CO_2CH_3), 3.85 (s, 3, OCH_3), 4.23 (q, 2, CH_2CH_3), and 6.85–7.30 ppm (m, 3, Ph).

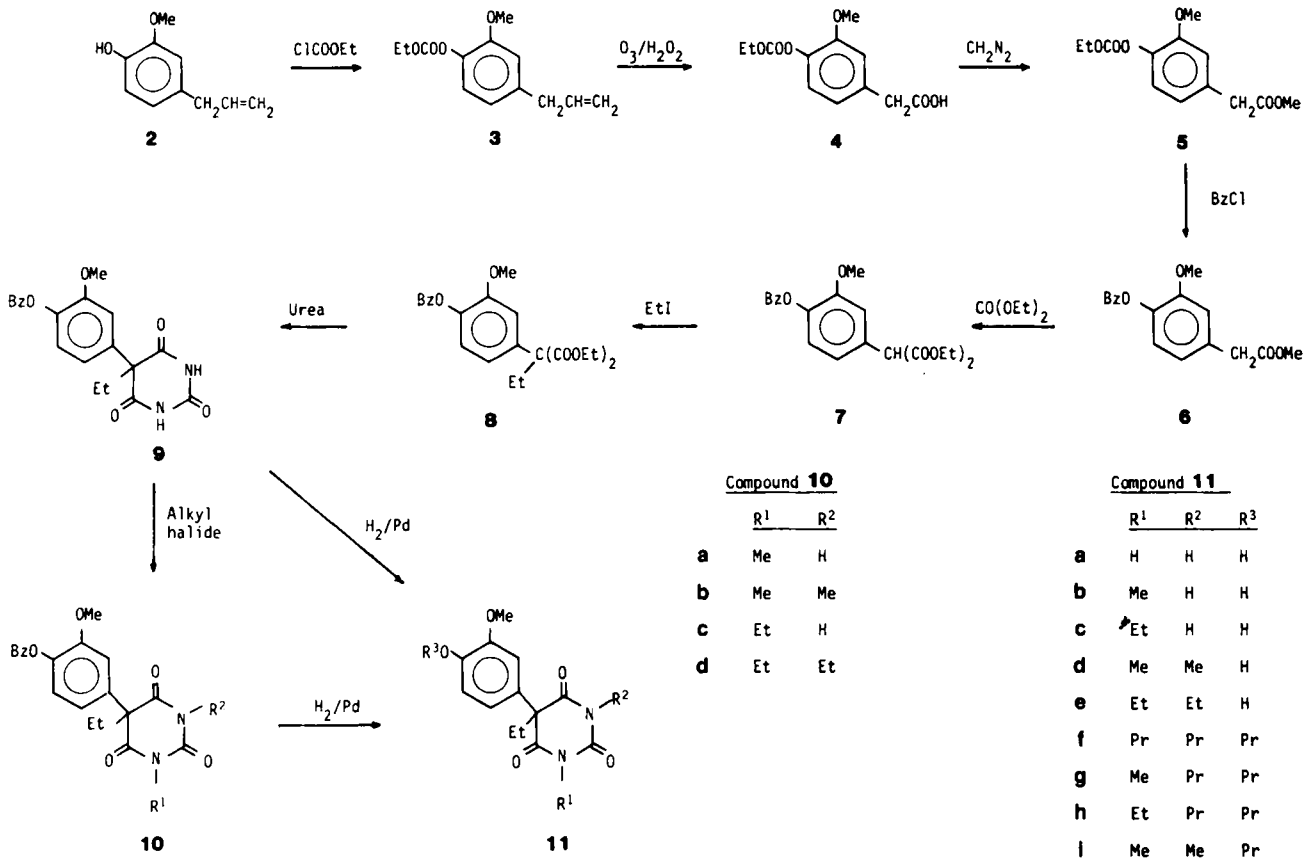
Anal.—Calc. for $C_{13}H_{16}O_6$: C, 58.2; H, 5.9. Found: C, 58.4; H, 6.0.

Methyl 4-benzyloxy-3-methoxyphenylethanoate (6)—Benzyl chloride (4.0 g, 32.0 mmol) and potassium carbonate (2.0 g, 14.5 mmol) were added to a solution of **5** (7.6 g, 27.0 mmol) in dry methanol (60 mL), and the mixture was refluxed for 20 h. The reaction mixture was evaporated, the residual oil was redissolved in ether (180 mL), and the extract was washed with water (50 mL), dried (anhydrous Na_2SO_4), and evaporated to give the benzyloxy derivative **6** (5.3 g, 65%). Compound **6** was recrystallized from hexane, mp 64–66 °C (lit.[9] 66–69 °C); MS: $M^{+\cdot}$ 286; ^1H NMR ($CDCl_3$): δ 3.61 (s, 2, CH_2), 3.70 (s, 3, CO_2CH_3), 3.81 (s, 3, OCH_3), 5.17 (s, 2, OCH_2), 6.58–6.80 (m, 3, Ph), and 7.21–7.40 ppm (s, 5, Ph).

Diethyl 4-benzyloxy-3-methoxyphenylmalonate (7)—Small pieces of sodium (0.30 g, 13 mmol) were added to a solution of **6** (3.4 g, 12 mmol) in diethyl carbonate (30 mL) and the mixture was heated under reflux for 1 h. Excess diethyl carbonate was removed under reduced pressure, and the residue was diluted with cold water (30 mL), neutralized with glacial acetic acid, and extracted with ether (3 × 50 mL). The extract was dried ($MgSO_4$) and evaporated, and the residual oil was distilled under reduced pressure to give **7** (bp 208–210 °C at 0.2 mm Hg) as a yellow oil (3.68 g, 82%); MS: $M^{+\cdot}$ 372; ^1H NMR ($CDCl_3$): δ 3.02 (t, 6, CH_3), 3.90 (s, 3, OCH_3), 4.10 (q, 4, CH_2), 4.49 (s, 1, CH), 5.19 (s, 2, OCH_2), 6.88–7.10 (m, 3, Ph), and 7.38–7.50 ppm (s, 5, Ph).

Anal.—Calc. for $C_{21}H_{24}O_6$: C, 67.7; H, 6.5. Found: C, 67.8; H, 6.6.

Diethyl 4-benzyloxy-3-methoxyphenylethylmalonate (8)—A 50% sodium hydride–oil dispersion (0.25 g, equivalent to 5.2 mmol of NaH) was washed with hexane and added to dry dimethylformamide (10 mL). Compound **7** (1.8 g, 5.0 mmol) in dimethylformamide (10 mL) was added to the sodium hydride suspension and the mixture was stirred until the evolution of hydrogen had ceased. Ethyl iodide (4.0 g, 25 mmol) was added in small portions over a period of 30 min, and the mixture was stirred at 25 °C for a further 16 h. The reaction mixture was diluted with water (40 mL) and extracted with ether (3 × 30 mL). The ethereal extracts were dried ($MgSO_4$) and concentrated, and the resulting oil was distilled under reduced pressure to give **8** as a yellow oil (1.67 g, 83.5%), bp 210–213 °C at 0.4 mm Hg; MS:



Compound **10**

	R^1	R^2
a	Me	H
b	Me	Me
c	Et	H
d	Et	Et

Compound **11**

	R^1	R^2	R^3
a	H	H	H
b	Me	H	H
c	Et	H	H
d	Me	Me	H
e	Et	Et	H
f	Pr	Pr	Pr
g	Me	Pr	Pr
h	Et	Pr	Pr
i	Me	Me	Pr
j	Et	Et	Pr

Scheme I—*Synthetic route for the O-methylcatechol metabolites of phenobarbital and its N-alkyl derivatives. Compounds 11f–j are not part of the synthetic route, but are analytical derivatives prepared for the GC–MS studies.*

$M^{+\cdot}$ 400; ^1H NMR ($CDCl_3$): δ 0.95 (t, 3, CH_2CH_3), 1.28 (t, 6, CH_3), 2.42 (q, 2, CH_2CH_3), 3.90 (s, 3, OCH_3), 4.20 (q, 4, CH_2), 5.11 (s, 2, OCH_2), 6.88–7.10 (m, 3, Ph), and 7.38–7.50 ppm (s, 5, Ph).

Anal.—Calc. for $C_{23}H_{28}O_6$: C, 69.0; H, 7.0. Found: C, 69.0; H, 7.1.

5-(4-Benzyloxy-3-methoxyphenyl)-5-ethylbarbituric acid (9)—A mixture of 8 (2.0 g, 5.0 mmol), urea (1.5 g, 25.0 mmol), and potassium *t*-butoxide (1.13 g, 10.0 mmol) in dimethyl sulfoxide (30 mL) was stirred at room temperature overnight. The mixture was diluted with cold water (20 mL) and extracted with ether (30 mL). The remaining aqueous layer was adjusted to pH 2 with 6 M HCl and extracted with ether (3 × 75 mL). The combined ether extracts were dried ($MgSO_4$) and then concentrated to yield a waxy solid. The solid was recrystallized from an ethyl acetate–hexane mixture to give 9 as a white solid (0.50 g, 27.2%), mp 238–240 °C; MS: $M^{+\cdot}$ 368.

Anal.—Calc. for $C_{20}H_{20}N_2O_5$: C, 65.2; H, 5.4; N, 7.6. Found: C, 65.3; H, 5.7; N, 7.6.

5-(4-Benzyloxy-3-methoxyphenyl)-5-ethyl-1-methylbarbituric acid (10a) and 5-(4-Benzyloxy-3-methoxyphenyl)-5-ethyl-1,3-dimethylbarbituric acid (10b)—Compound 9 (1.26 g, 3.4 mmol) was added to a solution of sodium (0.8 g, 3.5 mmol) in dry methanol (10 mL), followed by the addition of methyl iodide (0.5 g, 3.5 mmol). The resultant mixture was refluxed for 20 min and cooled, and the solvent was removed under reduced pressure at 40 °C. The oily residue was triturated with cold water (10 mL) and the mixture was extracted with ether (3 × 40 mL). The ether extracts were dried ($MgSO_4$), the ether was evaporated, and the residue was redissolved in a minimum of chloroform and then chromatographed on silica gel (60, 70–230 mesh) using distilled chloroform as an eluant. The first solid eluted from the column was recrystallized from methanol to yield white crystals of **10b** (0.29 g, 24%), mp 108–110 °C; MS: $M^{+\cdot}$ 396.

Anal.—Calc. for $C_{22}H_{24}N_2O_5$: C, 66.7; H, 6.1; N, 7.1. Found: C, 66.7; H, 6.2; N, 7.0.

The second component eluted from the column was also recrystallized from methanol as a white crystalline solid **10a** (0.46 g, 40%), mp 160–162 °C; MS: $M^{+\cdot}$ 382.

Anal.—Calc. for $C_{21}H_{22}N_2O_5$: C, 65.9; H, 5.8; N, 7.3. Found: C, 65.6; H, 5.8; N, 7.1.

A third component recovered from the column was shown to be the starting material 9 (0.22 g, 20%).

5-(4-Benzyloxy-3-methoxyphenyl-1,5-diethylbarbituric acid (10c) and 5-(4-Benzyloxy-3-methoxyphenyl)-1,3,5-triethylbarbituric acid (10d)—Ethyl iodide (0.76 g, 5.3 mmol) was added to 9 (1.5 g, 4.1 mmol) dissolved in a freshly prepared solution of sodium ethoxide (sodium, 0.10 g, 4.3 mmol in ethanol, 10 mL). The reaction mixture was refluxed for 45 min and cooled, and the solvent was removed under reduced pressure at 40 °C. The residual yellow oil was triturated with cold water (10 mL), extracted into ether (3 × 40 mL), and dried over $MgSO_4$. The ether was evaporated off and the residue was redissolved in a minimum of chloroform and chromatographed on silica gel (60, 70–230 mesh) using distilled chloroform as an eluant. The first component isolated from the column was an oil which solidified and was recrystallized from ethanol to give white crystals of **10d** (0.17 g, 10%), mp 105–106 °C; MS: $M^{+\cdot}$ 368.

Anal.—Calc. for $C_{24}H_{28}N_2O_5$: C, 67.9; H, 6.6; N, 6.6. Found: C, 67.8; H, 6.7; N, 6.4.

The second component after recrystallization from methanol gave a white solid **10c** (0.32 g, 20%), mp 148–150 °C; MS: $M^{+\cdot}$ 396.

Anal.—Calc. for $C_{21}H_{24}N_2O_5$: C, 65.8; H, 6.2; N, 6.8. Found: C, 65.6; H, 6.3; N, 7.2.

A final component (0.6 g, 40%) was unchanged starting material 9.

General Hydrogenation Procedure for 5-Ethyl-5-(4-hydroxy-3-methoxyphenyl) barbituric acid (11a)—Compound 9 (0.2 g, 0.5 mmol) in methanol (40 mL) was hydrogenated over 10% Pd/C catalyst (20 mg). When the uptake of hydrogen had ceased, the reaction mixture was filtered through celite, the filtrate was evaporated to dryness, and the solid was recrystallized from aqueous methanol to give white crystals of **11a** (0.12 g, 79%), mp 210–212 °C.

By employing this general procedure, all the benzylated barbiturates were hydrogenated to the corresponding hydroxy derivatives. The results are given in Table I.

Results

Urinary Metabolites of Phenobarbital—The gas chromatographic profile (total ion chromatogram) for the propylated extract of a urinary specimen from one subject (12–24 h after dosing with 300 mg of phenobarbital orally) is shown in Figure 1A. Many of the peaks are not drug related, but peak 1 is phenobarbital, peak 2 is *p*-hydroxyphenobarbital (as their respective propyl derivatives), and peak 3, which is scarcely observable in the chromatogram, has been identified as the tri-propyl derivative (**11f**) of the *O*-methylcatechol metabolite of phenobarbital. The presence of this compound is better illustrated in Figure 1B, which shows the specific ion chromatogram for *m/z* 405, the protonated molecular ion of the propylated derivative (**11f**). For comparison of both retention time and mass spectral features, the chromatogram of the synthetic standard (**11a**), derivatized in the same manner, is shown in Figure 1C. Figure 2A shows the partial

Table I—Analytical and Spectral Data for Synthesized *O*-Methylcatechol Metabolites

Compound	Yield, %	mp, °C	^1H NMR (CF_3COOH, Me_4Si)	$M^{+\cdot}$	Molecular Formula	Microanalysis			
						Found, %		Required, %	
11a	79	210–212	δ 0.85 (t, *J* = 7 Hz, 3), 2.32 (q, *J* = 7 Hz, 2), 3.72 (s, 3), 6.61–6.92 (m, 3).	278	$C_{13}H_{14}N_2O_5$	C H N	55.8 5.1 10.4	C H N	56.1 5.1 10.1
11b	80	169–170	δ 0.88 (t, *J* = 7 Hz, 3), 2.44 (q, *J* = 7 Hz, 2), 3.31 (s, 3), 3.81 (s, 3), 6.51–6.87 (m, 3).	292	$C_{14}H_{16}N_2O_5$	C H N	57.3 5.4 9.5	C H N	57.5 5.5 9.6
11c	87	129–130	δ 0.82 (t, *J* = 7 Hz, 3), 1.18 (t, *J* = 7 Hz, 3), 2.37 (q, *J* = 7 Hz, 2), 3.73 (s, 3), 3.91 (q, *J* = 7 Hz, 2), 6.61–6.99 (m, 3).	306	$C_{15}H_{18}N_2O_5$	C H N	58.6 6.0 9.0	C H N	58.8 5.9 9.2
11d	85	153–154	δ 0.77 (t, *J* = 7 Hz, 3), 2.31 (q, *J* = 7 Hz, 2), 3.23 (s, 6), 3.72 (s, 3), 6.41–6.83 (m, 3).	306	$C_{15}H_{18}N_2O_5$	C H N	58.5 6.0 9.0	C H N	58.8 5.9 9.1
11e	75	95–96	δ 1.19 (t, *J* = 7 Hz, 3), 1.45 (t, *J* = 7 Hz, 6), 2.79 (q, *J* = 7 Hz, 2), 3.75 (s, 3), 4.18 (q, *J* = 7 Hz, 2), 4.33 (q, *J* = 7 Hz, 2), 6.93–7.38 (m, 3).	334	$C_{17}H_{22}N_2O_5$	C H N	60.8 6.7 8.2	C H N	61.1 6.6 8.4

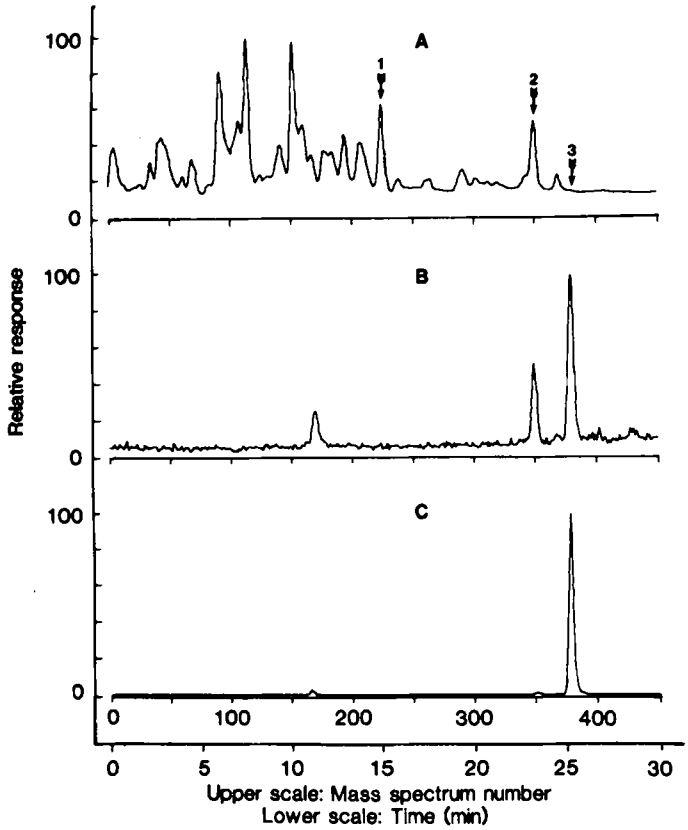


Figure 1—*Chemical ionization GC–MS data showing* (A) *total ion chromatogram for propylated extract of urine sample from subject who took phenobarbital (300 mg); drug-related peaks are indicated by the numbered arrows;* (B) *specific ion chromatogram for m/z 405 for the same urine sample as shown in* (A); *and* (C) *total ion chromatogram for propylated synthetic O-methylcatechol of phenobarbital.*

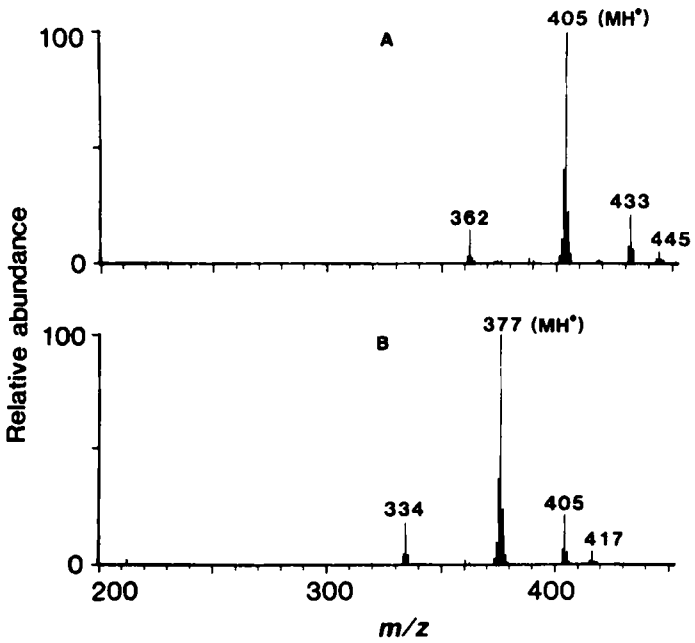


Figure 2—*Partial mass spectra (CI) for the propyl derivatives of the synthetic O-methylcatechols of phenobarbital* (A) *and mephobarbital* (B). *Panel* (A) *shows spectrum no. 382 from chromatogram* (C) *in Figure 1. Panel* (B) *shows spectrum no. 353 from chromatogram* (D) *in Figure 3. Identical spectra were obtained when appropriate urine extracts were studied by GC–MS (not shown).*

mass spectrum (CI) for synthetic *O*-methylcatechol of phenobarbital after propylation (**11f**); only the molecular ion cluster and adducts, and a fragment ion resulting from the loss of 43 amu from the protonated molecular ion, are prominent. Identical spectral features were obtained from the urinary extract, but with much lower signal:noise ratio (not shown).

Urinary Metabolites of Mephobarbital—The total ion chromatogram for the propylated extract of a urinary specimen from one subject (5–12 h after dosing with 300 mg of mephobarbital) is shown in Figure 3A. Phenobarbital (peak 1), *p*-hydroxyphenobarbital (peak 2), and the *O*-methylcatechol of phenobarbital (peak 3) are all recognizable at the same retention times as in Figure 1A. Other drug-related peaks are mephobarbital itself (peak 4, trace quantity only), *p*-hydroxymephobarbital (peak 5), and the derivatized *O*-methylcatechol of mephobarbital (**11g**, peak 6) which is not resolved from *p*-hydroxyphenobarbital. Figure 3B shows the specific ion chromatogram for *m/z* 377, the protonated molecular ion of the propyl derivative (**11g**) of the *O*-methylcatechol of mephobarbital, corresponding to the retention time of peak 6. The second response for this ion (scan 322) shows an

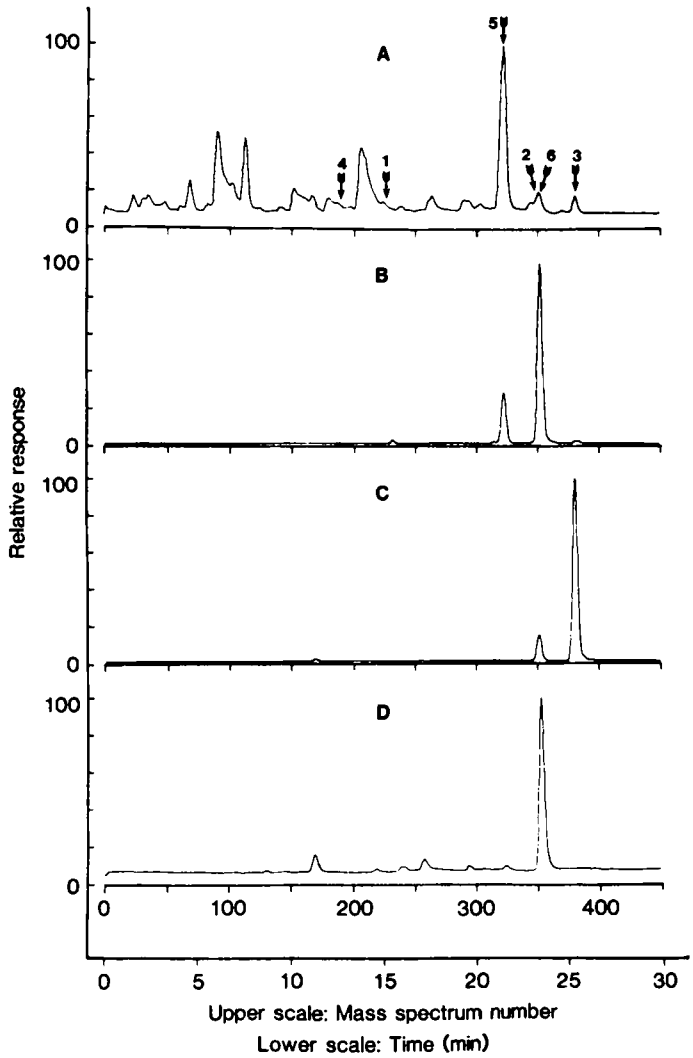


Figure 3—*Chemical ionization GC–MS data showing* (A) *total ion chromatogram for propylated extract of urine sample from subject who took mephobarbital (300 mg); drug-related peaks are indicated by the numbered arrows;* (B) *specific ion chromatogram for m/z 377 for the same urine sample as shown in* (A); (C) *specific ion chromatogram for m/z 405 for the same urine sample; and* (D) *total ion chromatogram for propylated synthetic O-methylcatechol of mephobarbital.*

adduct ion in the spectrum of *p*-hydroxymephobarbital (peak 5). The profile for *m/z* 405 is shown in Figure 3C, which confirms the presence of the *O*-methylcatechol of phenobarbital (compare with Figure 1C). Figure 3D shows the total ion chromatogram for the synthetic *O*-methylcatechol of mephobarbital (after propylation to give **11g**). The partial mass spectrum (CI) for the propyl derivative of the synthetic *O*-methylcatechol of mephobarbital is presented in Figure 2B. The similarities to Figure 2A are evident. A virtually identical spectrum was obtained from the urine specimen at scan no. 353, after allowing for the ions from chromatographically unresolved *p*-hydroxyphenobarbital.

***O*-Methylcatechols of Alkyl Barbiturates**—The *O*-methylcatechol metabolites (**11c**, **11d**, and **11e**) derived from ethylphenobarbital, dimethylphenobarbital, and diethylphenobarbital, respectively, were all found in urine samples from subjects given the respective barbiturates. Their corresponding propyl derivatives (**11h**, **11i**, and **11j**) showed mass spectra analogous to those illustrated in Figure 2; the protonated molecular ions for **11h**, **11i**, and **11j** are at *m/z* 391, 349, and 377, respectively (data not illustrated). As with mephobarbital, dealkylated metabolites were recognizable from each of these substrates. Results of quantification of these metabolites in urine over a period of 10 d in two volunteers following single 300-mg oral doses of the drugs are shown in Table II.

Discussion

Several drugs which undergo biotransformation to so-called *p*-hydroxy metabolites have been shown to yield 4-hydroxy-3-methoxy derivatives (*O*-methylcatechols) as additional minor biotransformation products. These include glutethimide,[10] phenytoin,[11] and ethotoin,[12] for which the identities of the metabolites have been confirmed by unequivocal synthesis. We previously obtained some presumptive evidence for the occurrence of *O*-methylcatechol metabolites of both phenobarbital and mephobarbital in urine following administration of mephobarbital to humans.[6] That suggestion was based solely on mass spectral evidence, and we noted that confirmation by synthesis was being attempted. In the present paper, the synthetic confirmation is presented, and we have expanded significantly on our original intention by extending the study to phenobarbital and further alkyl derivatives of phenobarbital, synthesized[7] for use in metabolic structure–activity studies. Thus, phenobarbital is metabolized to the *O*-methylcatechol **11a**. Each of the *N*-alkyl derivatives of phenobarbital yields the corresponding *O*-methylcatechol (**11b–e**), as well as those resulting from *N*-dealkylation.

Both phenobarbital and mephobarbital have been shown previously to give rise to a 3,4-diol metabolite and a 3,4-dihydrodiol metabolite,[5] but to our knowledge, the *O*-methylcatechols have not been previously reported, other than in our own study of mephobarbital noted earlier.[6] The mechanism of formation of diol metabolites has been the subject of recent study. Catechols can be formed either by two consecutive hydroxylations or by dehydrogenation of an intermediate dihydrodiol. Phenytoin has been shown in some elegant ^{18}O-incorporation studies[13] to be converted primarily by two consecutive hydroxylations rather than by dehydrogenation of the dihydrodiol intermediate, such as occurs for other model substrates (e.g., bromobenzene[14]). The aromatic diol is then partially converted to the *O*-methylcatechol by the enzyme catechol-*O*-methyltransferase (COMT).[15] A similar mechanism may be involved in the synthesis of the *O*-methylcatechols of the barbiturates reported here, though this awaits confirmation.

The assignment of the 4-hydroxy-3-methoxy structure to the metabolite rests on the identity of its chromatographic retention time and mass spectrum with those of the reference compound obtained by unequivocal synthesis. The only case among the drugs listed above as giving an *O*-methylcatechol metabolite for which the 3-hydroxy-4-methoxy positional isomer was also synthesized is glutethimide.[10] This isomer was shown to have different gas chromatographic retention characteristics on three stationary phases, compared with the 4-hydroxy-3-methoxy isomer. Although we were unable to prepare the 3-hydroxy-4-methoxy isomers for these barbiturate metabolites, it is most unlikely that they would cochromatograph with the 4-hydroxy-3-methoxy compounds.

The data reported in this paper were from studies in two volunteers. The occurrence of the same *O*-methylcatechol metabolite has been demonstrated in four additional volunteers. Even with the inclusion of these metabolites, the overall recovery of a dose of phenobarbital or mephobarbital remains quite low.[6,16] The time course of the excretion of the *O*-methylcatechols in urine has been defined in some detail. Concentrations in most cases become undetectably low after 48 h. Within this period, we have estimated that ~1% of the phenobarbital dose and 5% of the mephobarbital dose are eliminated as these metabolite(s). For the *N,N'*-dimethyl derivative, ~6% of dose was eliminated as total *O*-methylcatechols.

It should be noted that the mono-alkyl derivatives, mephobarbital and ethylphenobarbital, have a chiral center at C_5. It has been shown that only the *R*-enantiomer of mephobarbital is a substrate for aromatic hydroxylation.[17] One would anticipate, by analogy, that the metabolically formed *O*-methylcatechols may well be the *R*-enantiomers, but we have no proof of that. Similarly, the genetic polymorphism which applies to the metabolism of mephobarbital[17] may apply also to the *O*-methylcatechol formation, though its quantitative importance would be much less in this case.

Table II—Cumulative Urinary Recovery of *O*-Methylcatechols in Two Volunteers[a]

Compound Administered	Metabolite	Cumulative Percent of Dose	
		Volunteer 1	Volunteer 2
1a	**11a**	0.7	0.4
1b	**11b**	1.5	2.3
	11a	2.8	3.9
1e	**11c**	1.2	1.2
	11a	0.04	0.07
1f	**11d**	0.02	0.03
	11b	2.6	2.4
	11a	3.6	2.6
1g	**11e, 11c, 11a**	Identified but not quantified	

[a] Determined over a 10-d period following a 300-mg oral dose.

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