Table III—Benzoyl Peroxide Assay of Stability Samples of Benzoyl Peroxide Gel by HPLC and USP Methods

Stability Parameters	HPLC Method			USP Iodometric Method[a]	
	Benzoyl Peroxide, %	Percent Initial	Benzoic Acid, %	Benzoyl Peroxide, %	Percent Initial
Initial	5.77	—	—	5.39	—
60°, 2 days	5.64	97.7	0.20	5.38	99.8
60°, 4 days	5.48	95.0	0.27	5.20	96.5
60°, 7 days	5.34	92.5	0.44	5.00	92.8
60°, 9 days	5.30	91.9	0.48	4.90	90.9

[a] Recommended method for benzoyl peroxide lotions (5).

Nine analyses of a product sample[11] of gel containing 10% benzoyl peroxide by the HPLC method with Column 1 gave results with a relative standard deviation of 0.82%. Similarly, three analyses of the gel containing 5% benzoyl peroxide gave a relative standard deviation of 1.2%. Analysis of the lotion samples containing 4% benzoyl peroxide led to assay values with a relative standard deviation of 0.67%.

Although the precision and accuracy data of the HPLC method were collected using Column 1, the method was as good or better with Column 2. Five analyses of synthetic benzoyl peroxide gel samples using Column 2 gave recoveries of 99.9, 99.5, 99.1, 100.7, and 99.7% (average of 99.8%). Similarly, five analyses of the gel containing 5% benzoyl peroxide gave results with a relative standard deviation of 0.65%.

Application to Stability Studies—Samples of benzoyl peroxide gel were force degraded at 60°. Samples were taken at different time intervals and assayed by the HPLC method with Column 2. For comparison, the samples were also assayed by the USP iodometric method for benzoyl peroxide lotions (Table III).

Both the HPLC and the USP iodometric methods were stability indicating (Table III). Judging from the percent initial values of benzoyl peroxide obtained by both methods, the HPLC method showed only slightly higher degradation of samples. Excellent agreement between the amount of benzoyl peroxide degraded and the amount of benzoic acid formed was obtained with the HPLC method, which suggests that benzoic acid is the only, or the major, degradation product of benzoyl peroxide under the experimental conditions.

Comparison of the HPLC and USP iodometric methods (Table III) showed that the results (percent benzoyl peroxide) obtained by the USP method were consistently lower. To explain this difference, the accuracy of the USP method was evaluated using synthetic benzoyl peroxide gel samples. Analysis of three samples gave recovery values of 90.9, 90.1, and 86.6%. Evidently, the USP iodometric method for benzoyl peroxide lotion is not suitable for the benzoyl peroxide gel tested. Although the USP method appeared to be stability indicating, as judged from the percent initial values, its poor accuracy makes its use questionable for this gel.

Assay of five synthetic samples of benzoyl peroxide lotion by the USP method gave an average recovery value of 99.8%. Similar assay of five commercial benzoyl peroxide lotion samples gave a relative standard deviation of 0.83%. These results showed that the USP iodometric method for benzoyl peroxide lotions was accurate and precise. Limited comparative assay data of benzoyl peroxide lotion samples by the USP iodometric method and the HPLC method suggest that both methods are stability indicating when applied to lotions.

In summary, the HPLC method described is stability indicating for both gel and lotion formulations of benzoyl peroxide products; furthermore, the most likely degradation products or impurities, benzoic acid and perbenzoic acid, do not interfere in the assay. The method may be used with either type of octadecylsilane column. The more efficient Column 2, however, permits the simultaneous determination of benzoic acid.

[11] Similar composition to 5% benzoyl peroxide gel.

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Identification and Synthesis of a Methylated Catechol Metabolite of Glutethimide Isolated from Biological Fluids of Overdose Victims

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Abstract □ Urine samples from victims severely intoxicated by glutethimide were hydrolyzed enzymatically. TLC, GLC, and mass spectral analyses revealed a methylated catechol metabolite of the parent drug. Two synthetic pathways are described for the preparation of 2-ethyl-2-(3-methoxy-4-hydroxyphenyl)glutarimide and 2-ethyl-2-(3-hydroxy-4-methoxyphenyl)glutarimide. Comparisons of GLC and mass spectral data to a compound isolated from the body fluids of glutethimide overdose victims conclusively identified a new 3-methoxy-4-hydroxyphenyl metabolite of glutethimide in humans.

Keyphrases □ Glutethimide—metabolites identified in human urine, synthesized □ Metabolites—of glutethimide, identified in human urine, synthesized □ Sedatives—glutethimide, metabolites identified in human urine, synthesized

Since the introduction of glutethimide, (±)-2-ethyl-2-phenylglutarimide, in 1954 (1), it has been the subject of considerable study. Originally assumed to be a nonbarbiturate and sedative–hypnotic with few side effects

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(2), the drug rapidly gained popularity. However, reports of acute intoxication indicated that glutethimide possessed abuse potential (3). This drug is toxic in humans at doses considerably lower than those values previously determined from animal experiments (4).

BACKGROUND

Currently, toxicology studies as well as analyses of metabolites in both humans and animals are actively conducted to ascertain the reasons for the toxicity of glutethimide. A metabolite, 2-ethyl-2-phenyl-4-hydroxyglutarimide, was identified (5) in the blood of dogs (6) and patients intoxicated by glutethimide. This metabolite contributed to the morbidity and mortality of overdose victims (7); through biological assays in mice, the synthetic metabolite was shown to be as active as the parent drug in its sedative-hypnotic properties (8). Many other unusual metabolites of the parent drug also have been isolated and identified (8–10).

The existence of a previously unobserved human metabolite, 2-ethyl-2-(3-methoxy-4-hydroxyphenyl)glutarimide (XII), was proposed, and it was isolated (11). The designation of a substituted methylated catechol ring system was based initially on reports of similar metabolic pathways for aromatic rings (12) and on interpretation of mass spectral and NMR data. The information obtained from 100 μg of material isolated from the urine of an overdose victim was initially uncertain because of the other impurities and metabolites present. Interpretation of mass spectral data of the material revealed a tentative molecular ion at *m/e* 263, pointing to the addition of two oxygen atoms and one methylene group to the parent drug.

Initially, only a tentative conclusion could be reached from the NMR data. An NMR spectrum of the crude mixture revealed a singlet at δ 3.84 ppm, pointing to the presence of a methoxy group in the sample. From these limited data, a methylated catechol metabolite was proposed. Therefore, it was necessary to synthesize XII and 2-ethyl-2-(3-hydroxy-4-methoxyphenyl)glutamide (IV) to prove the structure of the proposed metabolite. The work reported here conclusively confirms the identity of a unique positional isomer as a new methylated catechol metabolite of glutethimide in humans.

EXPERIMENTAL

Sample Procurement—Urine samples were collected from three patients, soon after their admission to the hospital, who had taken large doses of glutethimide. The urine samples were collected and stored at −10° until the enzymatic hydrolysis.

Enzymatic Hydrolysis—Urine, 500-ml portions, was acidified to pH 5.5 with 1 *N* HCl. Citrate buffer, pH 5.5, was added to a total volume of 700 ml. An aqueous solution[1] of sulfatase and glucuronidase, 1.5 ml, then was added, and the mixture was incubated with shaking for 24 hr at 37°. The entire incubation mixture was shielded from light. The mixture then was extracted with 3 × 500 ml of ether[2]. After solvent evaporation, the dark extract was stored at 0° prior to preparative GLC.

GLC—After extraction, each enzymatically hydrolyzed sample was evaporated to a small volume. Then repetitive aliquots were injected into a preparative gas chromatograph[3], using a 1.5-m × 6.3-mm 15% OV-17 column[4] at 200°. Helium carrier gas at 40 ml/min yielded a variety of peaks which were individually collected. The purity of each collected fraction was monitored with an analytical gas chromatograph[5], using a 1.8-m × 3.1-mm 7% OV-17 glass column[4] at 200° with the flow rate of nitrogen carrier gas at 40 ml/min.

TLC—A small aliquot from each collected GLC peak was spotted on a 5 × 20-cm TLC plate[6] and developed with a standard drug-screening solvent system (ethyl acetate–methanol–ammonium hydroxide, 85:10:5). After the plate had developed 12 cm, it was removed and sprayed with mercuric sulfate and diphenylcarbazone[7] (0.5 g of mercuric oxide in 20 ml of concentrated sulfuric acid, diluted with 200 ml of water; 0.05% diphenylcarbazone in chloroform) to detect glutethimide-related materials as violet spots.

[1] Glusulase, Endo Laboratories, Garden City, NY 11530.
[2] Mallinckrodt Chemical Works, Montreal, Quebec, Canada.
[3] Model 90-P, Varian Aerograph, Walnut Creek, CA 94598.
[4] Applied Science Laboratories, State College, PA 16801.
[5] Model 2100, Varian Aerograph, Walnut Creek, CA 94598.
[6] Silica gel 60, E.M. Laboratories, Elmsford, NY 10523.
[7] Aldrich Chemical Co., Milwaukee, WI 53233.

Table I—Comparison of GLC Retention Times of Synthetic Intermediates and Analogs of Glutethimide on Various Columns Relative to Straight Chain Hydrocarbons as Methylene Units

Synthetic Compound	OV-1[a]	OV-17[b]	OV-210[c]
III	20.3	25.4	29.1
IV	24.0	29.2	32.1
VI	21.5	25.7	26.0
VII	21.1	25.1	25.4
VIII	22.3	26.9	29.5
IX	22.8	27.4	30.3
X	26.2	32.4	35.0
XI	28.5	34.5	35.5
XII	22.0	27.0	31.5

[a] Five percent on Gas Chrom Q (100–200 mesh); column, 1.8-m × 3.1-mm glass; temperature programmed, 6°/min from 175 to 260°; detector and injector temperature, 285°; nitrogen flow, 60 ml/min; hydrogen flow, 40 ml/min; and air flow, 300 ml/min. [b] Seven percent on Gas Chrom Q (100–200 mesh); column, 1.8-m × 3.1-mm glass; temperature programmed, 6°/min from 175 to 260°; detector and injector temperature, 285°; nitrogen flow, 60 ml/min; hydrogen flow, 40 ml/min; and air flow, 300 ml/min. [c] Three percent on Gas Chrom Q (100–200 mesh); column, 1.8-m × 3.1-mm glass; temperature, 200° isothermal; detector and injector temperature, 285°; nitrogen flow, 60 ml/min; hydrogen flow, 40 ml/min; and air flow, 300 ml/min.

Iodine vapor also was used to determine the total organics present on the plates. Synthetic materials were analyzed using silica gel plates. The solvent systems for development are noted with each sample.

NMR—Individual GLC fractions were dissolved in 75 μl of deuterated chloroform[8] (containing 1% tetramethylsilane) and placed in an NMR microbulb[9] sample holder. Each NMR spectrum was recorded on a medium-resolution NMR instrument[10].

Mass Spectrometry—A small aliquot of the analytically pure GLC fractions was subjected to 70-ev electron-impact mass spectrometry, using a single-focusing, magnetic sector[11], mass spectrometer interfaced to an analytical gas chromatograph[12]. Each collected GLC peak was either volatilized directly into the source of the mass spectrometer by probe distillation or analyzed directly by combined GLC–mass spectral techniques with a jet separator.

General Synthetic Methods—Melting points (uncorrected) were determined on a capillary apparatus[13]. IR spectra were recorded with a medium-resolution IR spectrophotometer[14] with sodium chloride cells and pressed potassium bromide pellets. Solutions were concentrated under reduced pressure using rotary evaporators[15]. GLC identification of synthetic materials utilized an analytical instrument[5] with a 1.8-m × 3.1-mm 3% OV-210 (Chromsorb W) column[4], a 3.05-m × 3.1-mm 3% Dexil-300 column[4], or a 1.8-m × 3.1-mm 7% OV-17 column[4]. Retention times were compared to straight chain hydrocarbon methylene units (Table I). Solvents were chromatographic grade.

2-Ethyl-2-(4-methoxyphenyl)glutarimide (III)—A solution of 2.0 g (8.6 mmoles) of 2-ethyl-2-(4-aminophenyl)glutarimide[16] (I) in 50 ml of 2 *N* H_2SO_4 was cooled to 5° in an ice water bath. To this solution was added slowly 1.2 g (17 mmoles) of sodium nitrite in 20 ml of water at 5°. The reaction mixture was stirred for 30 min until a clear solution developed. Then 0.54 g (8.8 mmoles) of urea was added to eliminate residual sodium nitrite in the reaction mixture. The diazonium salt, II, was added dropwise by a cannula to 250 ml of boiling methanol and then allowed to reflux for an additional 30 min.

The solution then was evaporated to dryness to yield an amber oil. Column chromatographic isolation [15 × 2.5-cm silica gel–cellulose (3:1) column, eluted with 160 ml of chloroform] yielded white crystals after solvent evaporation. Recrystallization from boiling ethanol and water yielded 1.2 g (54%) of III, mp 105–106°; TLC: R_f 0.70 [silica gel; chloroform–acetone (80:20), diphenylcarbazone–mercuric sulfate positive[6]]; IR (NaCl film): 3200 (NH), 1700, and 1680 (C=O, imide) cm^{-1}; mass spectrum: *m/e* 218 (100%), M^+ = 247 (75%).

2-Ethyl-2-(3-hydroxy-4-methoxyphenyl)glutarimide (IV)—A solution containing 0.15 g (0.61 mmole) of III and 5.8 ml of 30% H_2O_2[17]

[8] Silanor-C, Merck & Co., Rahway, NJ 07065.
[9] Wilmad Glass Co., Buena, NJ 08310.
[10] Model A-60, Varian Aerograph, Walnut Creek, CA 94598.
[11] Model 490-F, DuPont Instrument Product Division, Wilmington, DE 19898.
[12] Model 2700, Varian Aerograph, Walnut Creek, CA 94598.
[13] A. H. Thomas Co., Philadelphia, PA 19090.
[14] Model 4210, Beckman Instruments, Fullerton, CA 92634.
[15] Model RE/A, Brinkmann Instruments, Westbury, NY 11590.
[16] Generously supplied by Ciba-Geigy.
[17] Fisher Scientific Co., Fair Lawn, NJ 07410.

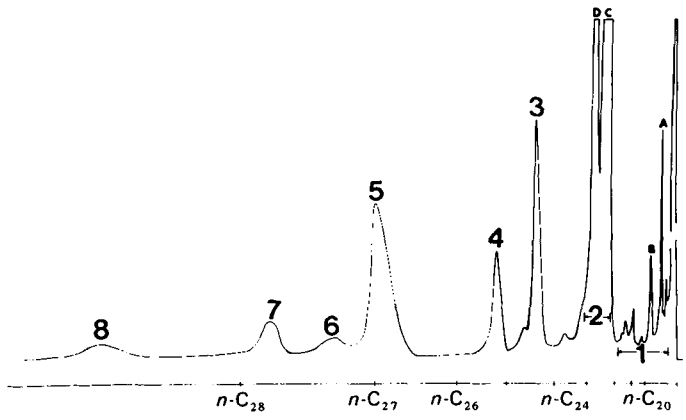


Figure 1—*Preparative GLC trace of an enzymatically hydrolyzed urine sample obtained from a glutethimide overdose victim after oral ingestion of 14 g of the parent drug. The ether extract of the sample was analyzed on a 1.8-m × 6.3-mm 7% OV-17 column at 200° with helium as the carrier gas (40 ml/min). The relative GLC retention times of* n-C_{20}-n-C_{28} *are indicated. Samples designated fractions 1–8 were collected for more complete characterization by other analytical techniques.*

dissolved in 30 ml of acetonitrile[18] was irradiated with 254-nm UV light[19] in a 50° water bath overnight. To the reaction mixture was added 10 ml of an aqueous solution saturated with sodium bisulfite to decompose the hydrogen peroxide. The phases were separated, and the aqueous layer was extracted with 3 × 50 ml of ether. The combined extracts were concentrated under reduced pressure, and the residue was streaked onto a 20 × 20-cm silica gel 60 TLC plate[6].

The residue was developed undisturbed to a distance of 15 cm. After solvent evaporation, 90% of the TLC plate was covered; only the edge (10%) was sprayed with diphenylcarbazone–mercuric sulfate[7] to reveal the imide positive band. The position of this band was extrapolated across the TLC plate, and then the remaining unreacted product was scraped off and extracted into methanol to yield IV; TLC: R_f 0.52 [silica gel; chloroform–acetone (80:20)]; IR (KBr): 3400 (NH), 1700 and 1600 (CO, imide), and 1200 (phenyl O) cm^{-1}; mass spectrum: M^+ = 263 (100%).

3-Methoxy-4-benzoxybenzyl Alcohol (VI)—A mixture of 10 g (65 mmoles) of vanillin alcohol[7] (V), 10 g (73 mmoles) of potassium carbonate, and 8.8 g (70 mmoles) of benzyl chloride[7] in 200 ml of ethanol was refluxed 12 hr. This solution was filtered hot. The filtrate was evaporated to dryness, yielding a brown oil, which solidified on cooling. Sublimation[20] resulted in 13.0 g (82.3%) of white crystals, mp 66–67°; IR (KBr): 3400 (OH), 3030, 1600, 1500 (phenyl), 1270, and 1130 (aromatic ether) cm^{-1}; mass spectrum: *m/e* 91 (100%), M^+ = 244 (12%); TLC: R_f 0.56 [silica gel; chloroform–acetone (80:20), sulfuric acid–char[21]].

3-Methoxy-4-benzoxybenzyl Chloride (VII)—Anhydrous hydrogen chloride gas[22] was bubbled through a solution of 11.5 g (47.1 mmoles) of VI in 150 ml of methylene chloride for 2 hr with stirring. The solvent was evaporated, yielding an amber oil, which solidified on cooling. Sublimation[20] produced 12 g (98%) of white crystals, mp 72–73°; IR (KBr): 3030, 1600, 1500 (phenyl), 1270, 1130 (aromatic ether), and 735 (chloride) cm^{-1}; mass spectrum: *m/e* 91 (100%), M^+ = 262/264 (3:1)(6%); TLC: R_f 0.84 [silica gel; chloroform–acetone (80:20), sulfuric acid–char[21]].

3-Methoxy-4-benzoxybenzylnitrile (VIII)—A solution of 6.5 g (25 mmoles) of VII and 10 g (154 mmoles) of potassium cyanide in 75 ml of dry dimethylformamide was stirred at room temperature for 12 hr. The solvent was evaporated, and the residue was partitioned between water and methylene chloride. Then the solvent was evaporated, producing a solid, which, upon recrystallization from hexane, yielded 3.9 g (62%) of white crystals, mp 69–70°; IR (KBr): 3030, 1600, 1500 (phenyl), 2230 (nitrile), 1270, and 1130 (aromatic ether) cm^{-1}; mass spectrum: *m/e* 91 (100%), M^+ = 253 (10%); TLC: R_f 0.38 (silica gel; benzene, sulfuric acid–char[21]).

2-(3-Methoxy-4-benzoxyphenyl)butyronitrile (IX)—This intermediate was generated when 10.5 g (41.5 mmoles) of VIII was added to a solution of 1.9 g (79 mmoles) of sodium hydride in dry toluene and stirred at room temperature for 2 hr. To this solution was added, dropwise, 4.6 g (42 mmoles) of ethyl bromide[7], and the resulting solution was refluxed for 17 hr. GLC peak area integrator[23] analysis of the resulting yellow oil showed 82% of IX, 10% of unreacted VIII, and 6% of a dialkylated product. This mixture was used without further purification; mass spectrum: *m/e* 91 (100%), M^+ = 281 (8%); TLC: R_f 0.5 (silica gel; benzene, sulfuric acid–char[21]).

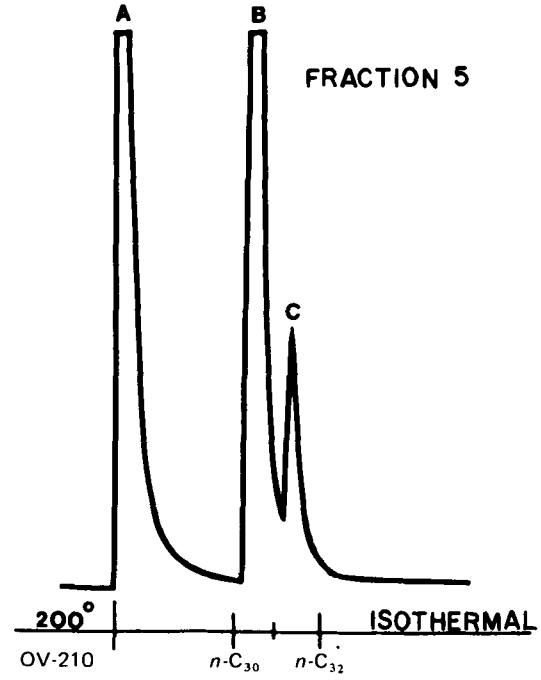


Figure 2—*Analytical GLC trace of metabolites (fraction 5) collected by preparative GLC. Although a single GLC peak was shown on OV-17, the OV-210 phase revealed two components: A, solvent; B, 2-ethyl-2-(4-hydroxyphenyl)glutarimide; and C, new metabolite, which was analyzed mass spectrometrically.*

2-Ethyl-2-(3-methoxy-4-benzoxyphenyl)glutarodinitrile (X)—To a solution of 4.7 g of the products from the previous reaction and 0.94 g (18 mmoles) of acrylonitrile[7] in dry dioxane[7] was added slowly, with stirring, 2.0 ml of 40% base[24]. The resulting solution was refluxed for 21 hr. The solvent was then evaporated, and the residue was extracted with ether to yield 4.6 g (82%) of X as white crystals, mp 125–126°; IR (KBr): 3030, 1600, 1500 (phenyl), 2230 (nitrile), 1270, and 1130 (aromatic ether) cm^{-1}; mass spectrum: *m/e* 91 (100%), M^+ = 334 (9%); TLC: R_f 0.08 (silica gel; benzene, sulfuric acid–char[21]).

2-Ethyl-2-(3-methoxy-4-hydroxyphenyl)glutarimide (XII)—A solution of 1.5 g (4.5 mmoles) of X and 7 ml of concentrated sulfuric acid in 25 ml of acetic acid was heated in a water bath for 6 hr. The resulting dark-brown liquid was poured onto 100 ml of crushed ice and extracted with methylene chloride. This organic fraction was washed well with saturated sodium carbonate (100 ml). The organic layer was extracted with ether and evaporated to dryness, yielding XI; TLC: R_f 0.67 [silica gel; chloroform–acetone (80:20), diphenylcarbazone–mercuric sulfate positive]; mass spectrum: *m/e* 91 (37%), 296 (53), 324 (62), and 353 (M^+, 100).

Compound XI was finally reduced with 10% palladium-on-charcoal[25] at 1 atmosphere in a Parr hydrogenator[26] to yield XII; TLC: R_f 0.52 [silica gel; chloroform–acetone (80:20), diphenylcarbazone–mercuric sulfate positive]; mass spectrum: *m/e* 91 (10%), 131 (30), 206 (53), 234 (100), and 263 (M^+, 85).

RESULTS AND DISCUSSION

In many acute drug intoxications, the analysis of biological fluids is of paramount importance for the identification of the causative toxic

[18] Mallinckrodt Chemical Co., St. Louis, MO 63147.
[19] Quartz pencil lamp, Edmund Scientific Co., Barrington, NJ 08007.
[20] Model 8027, Ace Glass Inc., Vineland, NJ 08360.
[21] Sulfuric acid–char indicates that the TLC plate was sprayed with 5% H_2SO_4–H_2O and heated to 100° for 2 min to reveal brown spots.
[22] Generated from concentrated sulfuric acid and sodium chloride.
[23] Model 480, Varian Aerograph, Walnut Creek, CA 94598.
[24] Triton B as the methoxy salt (40% in methanol), Baker Chemical Co., Phillipsburg, NJ 08865.
[25] Matheson, Coleman and Bell, Norwood, OH 45212.
[26] Parr Instrument Co., Moline, IL 61265.

agent. Routinely, body fluids from overdose victims can be extracted and the compounds of interest can be screened by TLC (13), analyzed by GLC (14), or quantitated by combined GLC–mass spectrometric techniques (15). Once the drugs responsible for the severe intoxication have been identified, proper supportive techniques for the patient can be administered.

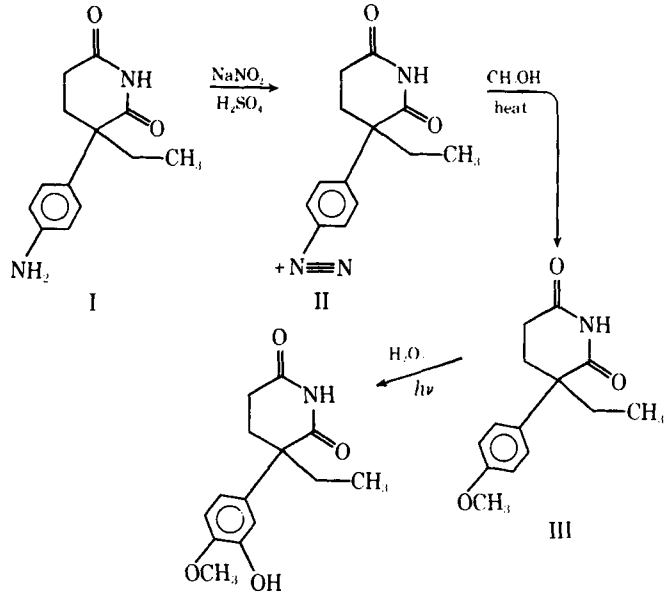
Severe glutethimide intoxication in drug overdose victims is easily identified because the levels of the intoxicant are usually high. Extractions and analysis of urine samples typically reveal the parent drug, which is easily identified by standard TLC and GLC methods (13). However, recent questions have been raised (6) concerning the impact of metabolites in the body fluids of overdose victims and their contribution to the morbidity and mortality associated with glutethimide overdose.

The preparative GLC trace of an enzymatically hydrolyzed urine extract obtained from a glutethimide overdose victim, identified as having taken 14 g of glutethimide, is seen in Fig. 1. From the diagram, eight major fractions were designated. Each fraction was collected repeatedly to obtain sizable quantities for complete characterization. By comparison of GLC retention times to authentic glutethimide, peak C of fraction 2 was considered initially to be the parent drug. However, following mass spectral analysis, peak C was determined to be glutethimide and α-phenylglutarimide, a known metabolite.

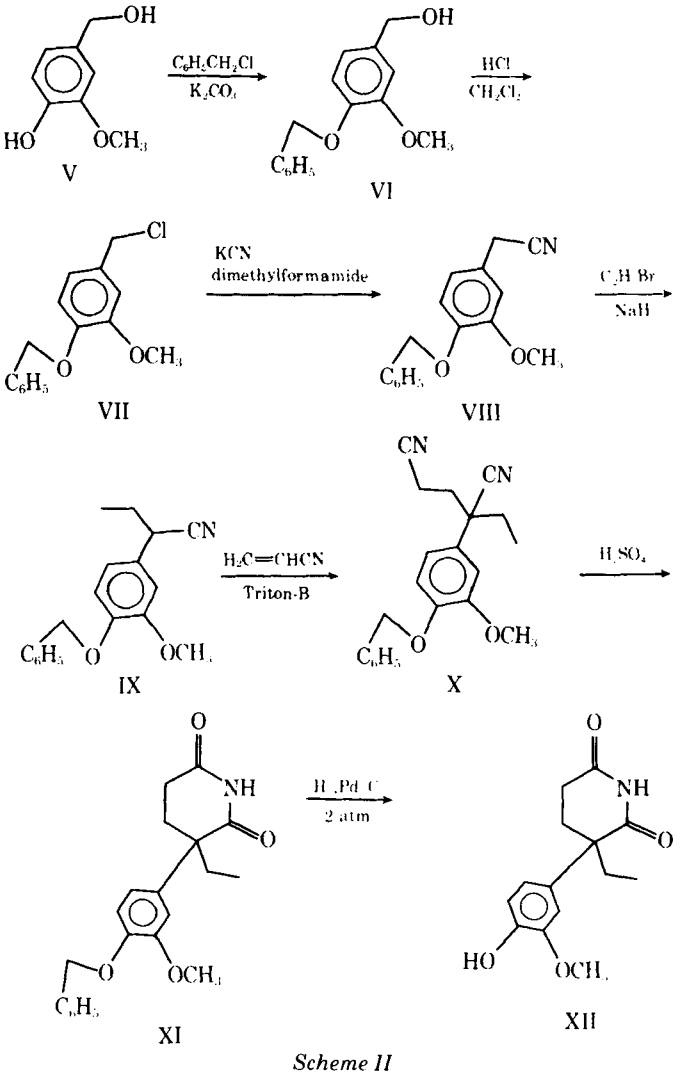
Because fraction 5 appeared to be reasonably pure and in high concentrations, it was next analyzed completely. However, mass spectral analyses revealed two components, which were easily separated when the GLC liquid support phase was changed from OV-17 to OV-210 (Fig. 2). The major component was identical in all respects to *p*-hydroxyphenylglutethimide, a known metabolite of the parent drug (16). The minor component appeared to be new and unique. The mass spectrum obtained from this compound is seen in Fig. 3A. A molecular ion at *m/e* 263, an intense ion at *m/e* 234 (indicating loss of an ethyl side chain), and the very polar GLC characteristics compared to the parent drug suggested a methylated catechol. Confirmation of a methoxy group was made by NMR analysis of fraction 5, a mixture, which revealed a signal at δ 3.84 ppm, often indicative of methoxy groups.

With this information, 10 possible isomeric methylated catechol metabolites of glutethimide could be proposed. However, in most metabolic pathways, 3-OH and 4-OCH_3, 3-OCH_3, or 4-OH phenyl substitution patterns predominate. For example, the methylated catechol metabolites of levodopa (17), phenacetylurea (18), and phenytoin (12) in humans have been identified and synthesized to confirm that the 3-OCH_3 and 4-OH phenyl metabolites are the rule and not the exception. Therefore, to make direct analytical comparisons, both 3,4-monomethylated catechol isomers of glutethimide were prepared. Schemes I and II reveal the synthetic pathway leading to IV and XII, respectively.

In Scheme I, I was converted to the diazonium salt, II, and hydrolyzed in absolute methanol (19) to yield a new *p*-methoxy analog, III. Following a modification of a known procedure (20), the new methylated catechol, IV, was conveniently prepared by photochemical oxidation of III in hydrogen peroxide.



Scheme I



Scheme II

To prepare XII, partial methylation of the catechol metabolite (21) of glutethimide was attempted. However, complex mixtures of products were obtained (with either diazomethane or methyl iodide in base) and were difficult to characterize accurately. Therefore, a total synthesis of XII was needed to assure the isomeric purity of the final product.

Scheme II reveals the complete synthesis leading to the desired compound. Protection of vanillin alcohol, V, with benzyl chloride yielded VI in high purity and in an excellent yield. Conversions of the alcohol, VI, to the chloride, VII, and then to the nitrile, VIII, were accomplished following procedures (22) similar to those described for the preparation of the parent drug. The monoethylated intermediate, IX, was generated by reacting ethyl bromide with VIII in a suspension of sodium hydride and refluxing toluene. The reaction was analyzed frequently by GLC to monitor the formation of IX as the ethyl bromide was added slowly to the reaction mixture. This procedure reduced the generation of excessive amounts of a diethylated product.

Conversion of IX to the dinitrile, X, was accomplished following known procedures (23). It was next determined that cyclization of X to the protected glutethimide analog, XI, could be performed in either strong acid or strong base. Base hydrolysis of X yielded a purer product, XI, which required only the addition of acid to the reaction mixture to precipitate the desired compound. However, the yields by this method were lower then those obtained by acid hydrolysis. Finally, catalytic hydrogenation of XI generated XII, which was isolated and characterized by standard methods (23).

Figures 3B and 3C show the mass spectra of IV and XII, respectively. The compound isolated from the enzymatically hydrolyzed glutethimide urine sample apparently produces a mass spectrum virtually identical to the spectrum in Fig. 3C. Whereas the base peak (100%) is *m/e* 263 (the

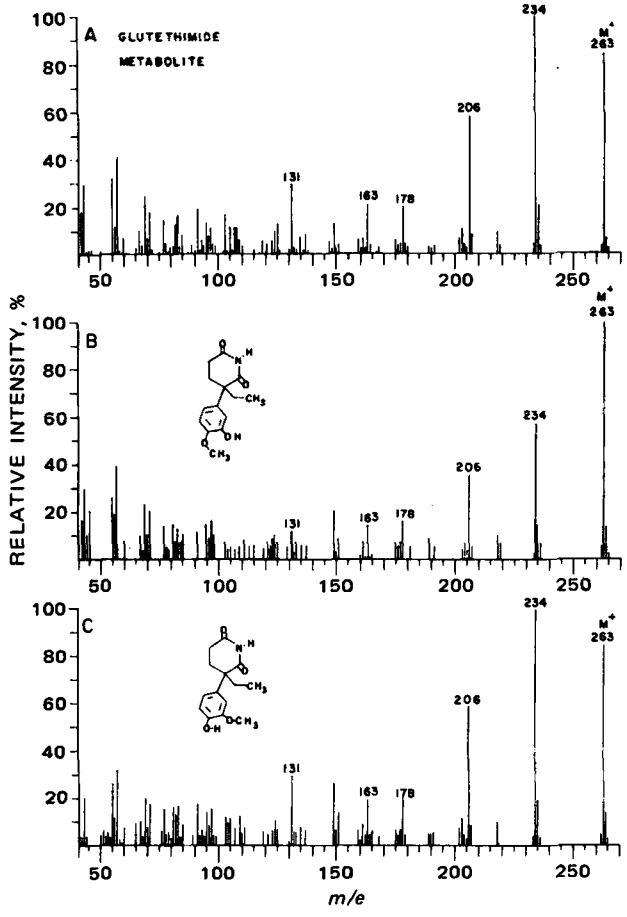
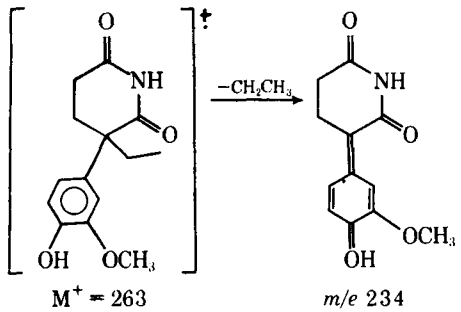


Figure 3—*Mass spectral comparison of the minor component (C in Fig. 2) to two methylated catechol analogs of glutethimide. The mass spectral data of the glutethimide metabolite (A) agree favorably with 2-ethyl-2-(3-methoxy-4-hydroxyphenyl)glutarimide (C).*

molecular ion) for IV, the glutethimide metabolite and the synthetic XII reveal a base peak (100%) for *m/e* 234 (loss of the ethyl side chain). The *p*-hydroxyphenyl compound more strongly favors stabilization of the benzyl cation (XIII, Scheme III) and is clearly an aid in differentiating the isomers.

These findings were confirmed by comparisons of GLC retention times. The metabolite (collected preparatively on OV-210 and reanalyzed) revealed methylene unit hydrocarbon retention values (24) of 22.0, 27.0, and 31.5 on OV-1, OV-17, and OV-210, respectively. Compound XII revealed exactly the same values. However, IV revealed methylene unit values of 24.0, 29.2, and 32.1 on OV-1, OV-17, and OV-210, respectively. Therefore, the monomethylated catechol metabolite of glutethimide is XII.

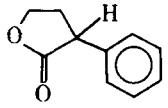
In Fig. 1, it can be seen that many other fractions were collected; each is currently in different phases of characterization. Fraction 1 possessed



$M^+ = 263$ *m/e* 234

XII XIII

Scheme III



XIV

two major components, A and B, which were initially identified by mass spectrometric techniques (25) and finally synthesized (26) to confirm *p*-hydroxymethylbenzoate and α-phenyl-γ-butyrolacetone (XIV), respectively. The former may be a metabolite of glutethimide, but the latter clearly was associated (25) with new metabolites of the parent drug.

Fraction 2-D revealed a compound whose mass spectral and GLC properties were identical to data previously reported (6) for 2-ethyl-2-phenyl-4-hydroxyglutarimide, an active metabolite of the parent drug.

Fractions 3 and 4 both possessed different chromatographic properties than the known hydroxylated metabolite, 2-ethyl-2-phenyl-4-hydroxyglutarimide, fraction 2-D. However, both compounds also revealed a molecular ion at *m/e* 233, indicative of a single O added to glutethimide, suggesting new hydroxylated metabolites. Currently, synthetic hydroxylated analogs of glutethimide are in preparation to confirm the exact structure of each compound.

Due to the limited amount of material present in fraction 6, only preliminary characterization was attempted. However, fraction 7 was shown by mass spectral analysis to possess a molecular weight of 233 and to produce a much different spectrum than that of the parent drug or metabolites. NMR data revealed the complete loss of the ethyl side chain, and TLC revealed a very polar compound. Thus, a new and very polar metabolite is proposed for peak 7.

The preliminary data obtained concerning peak 8 revealed a compound with a molecular weight of 279 (characterized by mass spectrometry), one methoxy group by NMR, and a compound more polar by TLC analysis than the 2-ethyl-2-(4-hydroxyphenyl)glutarimide (16) (fraction 5) and active toward mercuric sulfate–diphenylcarbazone (indicative of imide systems). Consequently, a dihydroxymethoxy metabolite of glutarimide is proposed. The exact substitution on the parent drug is not known, but synthetic work is in progress.

In conclusion, the glutethimide overdose victims described provided many new metabolites of the parent drug. Further characterization and synthesis of each individual metabolite are currently in progress. The results may uncover new and active metabolites that contribute to the toxicity associated with an overdose of the parent compound.

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Hydrolysis and Dissolution Behavior of a Prolonged-Release Prodrug of Theophylline: 7,7′-Succinylditheophylline

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Abstract □ The physicochemical characteristics of 7,7′-succinylditheophylline, a slow dissolving prodrug of theophylline, were investigated at 25°. One molecule of 7,7′-succinylditheophylline hydrolyzed to give two molecules of theophylline and one molecule of succinic acid. At 25° in aqueous solution, constant ionic strength, and zero buffer concentration, the pH profile for the hydrolysis of 7,7′-succinylditheophylline could be described adequately by a spontaneous constant, k_0, of 8.15×10^{-2} sec^{-1} and a hydroxide catalytic constant, k_{OH^-}, of 1.46×10^5 M^{-1} sec^{-1}. No specific acid catalysis was seen at pH values as low as 1. This dissolution rate of 7,7′-succinylditheophylline from a constant surface area pellet was independent of pH below 8. However, at pH values greater than 8, the dissolution rate was accelerated by a base. This behavior was consistent with the dissolution rate being catalyzed by the simultaneous chemical reaction of 7,7′-succinylditheophylline with hydroxide ion at a rate such that substantial hydrolysis of the substrate was occurring in the dissolution film. Dissolution with simultaneous chemical reaction for 7,7′-succinylditheophylline was analyzed theoretically on the basis of a film theory model. Excellent correlation between the theoretical and observed dissolution behavior was found. At pH values less than 8, the dissolution of 7,7′-succinylditheophylline was 35 times slower than that of theophylline. Based on this result, the aqueous solubility of 7,7′-succinylditheophylline was estimated to be 1.63×10^{-3} M.

Keyphrases □ 7,7′-Succinylditheophylline—hydrolysis and dissolution, effect of pH □ Prodrugs, theophylline—7,7′-succinylditheophylline, hydrolysis and dissolution, effect of pH □ Hydrolysis—7,7′-succinylditheophylline, effect of pH □ Dissolution—7,7′-succinylditheophylline, effect of pH □ Theophylline prodrugs—7,7′-succinylditheophylline, hydrolysis and dissolution, effect of pH

Theophylline is an effective bronchodilator used widely for the treatment of asthma. The improvement of pulmonary function in asthmatic patients was related to the plasma theophylline concentration (1–4). An optimal serum concentration range of 10–20 μg/ml was reported (5), although ranges from 5 to 20 μg/ml are often quoted. Theophylline levels greater than 16 μg/ml were associated with toxicity (5). For a drug with this narrow therapeutic index, it is difficult to maintain the optimum blood level for the desired time period; higher concentrations frequently result in toxicity and lower values appear less likely to provide maximal therapeutic benefit. Those problems suggest that modification of pharmaceutical properties are necessary to maintain the optimum blood level by the prodrug approach.

One main objective of a prodrug is to influence the plasma drug concentration–time profile with known pharmacological activity. This effect is achieved conveniently when the prodrug is much less soluble in water and has a slower dissolution rate in aqueous fluid than the parent drug. Under these circumstances, the appearance of the parent drug in the body is slowed by the slow dissolution of the prodrug in the GI tract (6–8). The purpose of this study was to investigate some physicochemical properties of 7,7′-succinylditheophylline as a potential prolonged-release theophylline prodrug.

THEORETICAL

The dissolution of 7,7′-succinylditheophylline will be shown to be pH dependent above pH 8. At pH values greater than 8, the hydrolysis of 7,7′-succinylditheophylline to theophylline occurs so rapidly that it takes place in the diffusion layer film. That is, the dissolution of 7,7′-succinylditheophylline occurs with a simultaneous irreversible chemical reaction.

Theoretical predictions of the dissolution rate with simultaneous irreversible chemical reactions have been treated previously (9, 10). The experimental data for the dissolution rate of 7-acetyltheophylline agreed with theoretical predictions based on the film theory model under steady-state conditions. This theoretical treatment also may be applied to 7,7′-succinylditheophylline.

The problem to be considered is that in which a solid phase, A, dissolves into the liquid phase and then reacts irreversibly with a species, B, already present in the liquid phase: $A + B \rightarrow C + D$. The intermediate species, D, then reacts irreversibly with B to form the final products: $D + (n-1)B \rightarrow$ products, where n is the number of molecules of B. If A is 7,7′-succinylditheophylline and B is hydroxide ion, n is equal to 2 below pH 8 and to 4 above pH 9. The first step, disappearance of A by reaction with B, would be considered a rate-determining step.

Let A be the concentration of 7,7′-succinylditheophylline at a distance X within the liquid film, t the time, k_0 the first-order rate constant of a

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