Identification of 2-Ethyl-2-methyl-3-hydroxysuccinimide as a Major Metabolite of Ethosuximide in Humans

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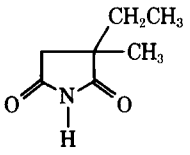
Abstract □ A previously unreported major urinary metabolite of the antiepileptic drug ethosuximide (I) was isolated from a patient suffering from petit mal epilepsy. The metabolite was identified as the ring-hydroxylated ethosuximide, 2-ethyl-2-methyl-3-hydroxysuccinimide (II), by GLC, low- and high-resolution electron-impact mass spectrometry, and proton NMR spectroscopy of its *N*-methyl derivative.

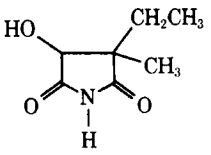
Keyphrases □ Ethosuximide—isolation and identification of 2-ethyl-2-methyl-3-hydroxysuccinimide as human urinary metabolite □ 2-Ethyl-2-methyl-3-hydroxysuccinimide—isolation and identification as major human urinary metabolite of ethosuximide □ Antiepileptic agents—isolation and identification of 2-ethyl-2-methyl-3-hydroxysuccinimide as major human urinary metabolite of ethosuximide

Ethosuximide (2-ethyl-2-methylsuccinimide, I) is the anticonvulsant drug of choice in the treatment of petit mal epilepsy. Two human urinary metabolites of this antiepileptic were recently (1) identified: 2-(1-hydroxyethyl)-2-methylsuccinimide (present as a diastereoisomeric pair) and 2-acetyl-2-methylsuccinimide (found only in cases of multiple dosing). Other metabolic products of I were indicated but not identified.

This paper reports the identification of a major human urinary metabolite of I, 2-ethyl-2-methyl-3-hydroxysuccinimide (II), employing the combined techniques of GLC, high- and low-resolution electron-impact mass spectrometry, and proton NMR spectroscopy[1].



I



II

EXPERIMENTAL

Isolation and Chemical Pretreatment of Urinary Extracts—Twenty-four-hour urine collections were obtained from: *(a)* a 24-year-old female patient, 60 kg, receiving 1000 mg/day for 7 years, *(b)* a 20-year-old male volunteer, 80 kg, receiving a 500-mg single oral dose, and *(c)* a 30-year-old male volunteer, 70 kg, receiving a 500-mg single oral dose. Aliquots (20 ml) of urine were acidified to about pH 1 with 6 *M* HCl, saturated with sodium chloride, and extracted with three 20-ml portions of ether. The combined ether extracts were evaporated to dryness at reduced pressure[2], and the residue was dissolved in 1 ml of methanol. The resulting solution was treated (2) with excess ethereal diazomethane for about 1 hr at room temperature, after which time the sample was concentrated to near dryness under an air jet. This residue was then dissolved in 200 μl of methanol for subsequent GLC analysis.

GLC and Mass Spectrometry—Analytical separations were performed by on-column injections using a dual-column gas chromatograph[3] equipped with dual hydrogen flame-ionization detectors. Stainless steel columns, 1.82 m (6 ft) × 0.31 cm (0.125 in.), packed with either 3% SE-30 or 3% OV-17 on Chromosorb G (80–100 mesh) were employed for separations under the following conditions: injector, 225°; detector, 310°; carrier gas (helium), 30 ml/min; and column oven, 100–300° at 8°/min. Relative retention times are expressed in terms of Kovats Indices (KI) (3). Preparative GLC was performed on the same instrument under the same conditions utilizing a splitting device designed so that about 5% of the effluent from the GLC column was directed to the detector and the remainder was directed to a collection device (4) *via* a stainless steel transfer line maintained at 225°.

Combined GLC–mass spectrometry was carried out under identical GLC conditions as already reported employing a single-column gas chromatograph[4] interfaced to a low-resolution, double-focusing mass spectrometer[5]. Half of the GLC column effluent was directed to a flame-ionization detector and the other half was directed to the ion source of the mass spectrometer *via* a stainless steel transfer line (230°) and a single-stage jet separator (260°). The following electron-impact mass spectrometric conditions were employed: ionizing voltage, 70 ev; ionizing current, 100 μamp; resolution, 1100 (10% valley); and ion source temperature, 280°.

High-resolution mass measurements were performed[6] employing the direct probe insertion technique under the following electron-impact mass spectrometry conditions: ionizing voltage, 70 ev; resolution, 10,000 (10% valley); ion source temperature, 70°; and direct probe temperature, 70°.

NMR Spectrometry[7]—Approximately 100 μg of the GLC isolated samples was dissolved in a stock solution of carbon tetrachloride, which was either saturated with perfluorocyclobutane or contained 5% by volume hexafluorobenzene and 0.1% by volume tetramethylsilane. The fluorocarbons served as the lock material for spectrometer frequency stabilization, and the tetramethylsilane served as the chemical shift reference material. Spectra were obtained in 5-mm diameter cylindrical microcavity (about 100 μl) sample cells.

RESULTS AND DISCUSSION

The GLC trace (OV-17) obtained upon injection of the methylated urine extract from a patient maintained on ethosuximide is presented in Fig. 1. Comparison of this tracing to similar extracts obtained from subjects not receiving ethosuximide indicated that the peaks at KI 1480, 1595, and 1625 result from drug administration. When the urine extract shown in Fig. 1 was not treated with diazomethane or was treated with diazomethane but without the

[1] While this manuscript was in preparation, another report appeared concerning the identification of II as a metabolite of ethosuximide by GLC–mass spectrometry; see M. G. Horning, C. Stratton, J. Nowlin, D. J. Harvey, and R. M. Hill, *Drug Metab. Dis.*, 1, 569(1973). Structure II was inferred from the mass spectrum of its *N*-methyl-*O*-trimethylsilyl ether derivative, but hydroxylation at the C-2 methyl group could not be conclusively ruled out.

[2] Roto-vap.

[3] Varian model 2700.

[4] Varian model 1200.

[5] DuPont 21-491.

[6] On an AEI MS-9 mass spectrometer.

[7] Proton NMR spectra were obtained at 90 MHz on a Bruker HX-90 high-resolution spectrometer equipped with a fast Fourier transform system employing a Nicolet 1083 computer.

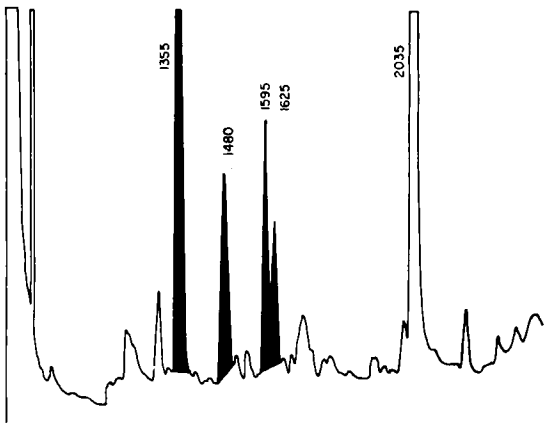


Figure 1—*Analytical GLC trace (OV-17) of methylated human urine extract from a female patient maintained on ethosuximide for 7 years. Relative retention times are expressed in terms of Kovats Indices. Shaded areas represent peaks due to ingestion of drug.*

added methanol catalyst, only one significant peak, at KI 1450, resulted upon subjection to identical GLC analysis. This peak was found to correspond to unmethylated ethosuximide by cochromatography (OV-17 and SE-30) and combined GLC-mass spectrometry.

The compound possessing KI 1355 (Fig. 1) was shown by cochromatography (OV-17 and SE-30) to be *N*-methylethosuximide. In addition, the electron-impact mass spectrum of this compound was identical to an authentic sample (Fig. 2). The spectrum is characterized by a weak but detectable molecular ion (M^+) at *m/e* 155 and a base peak (100% relative intensity) at *m/e* 127 (M − 28). Ring fission of the succinimide nucleus (5) gives rise to major fragment ions at *m/e* 70 and 55.

Figure 3 shows the mass spectrum obtained for KI 1480. Occurrence of a molecular ion (M^+) at *m/e* 171, 16 amu higher than that obtained from the methylated drug, suggests that the compound is a monohydroxylated derivative of I. The shift of 16 amu for each of the three major ions in the spectrum (*m/e* 143, 86, and 71) further supports the monohydroxyethosuximide structure. Isolation of this compound by preparative GLC and subsequent subjection to high-resolution mass spectrometry confirmed that *m/e* 171 does possess the $C_8H_{13}NO_3$ molecular formula (calc., 171.0895; found, 171.0901).

The intense peak at *m/e* 143 in the mass spectrum of this methylated metabolite was composed of a single ionic species of empirical formula $C_6H_9NO_3$ (calc., 143.0582; found, 143.0590). Formation of this ion represents loss of the elements of ethylene (C_2H_4) from the molecular ion, a fragmentation pathway favorable only if the integrity of the C-2 ethyl side chain of I is maintained. The mechanism by which this 143 ion occurs undoubtedly involves a McLafferty rearrangement, as previously proposed for similarly substituted cyclic imides (5) and barbiturate derivatives (6).

Table I—Proton NMR Chemical Shifts and Coupling Patterns of *N*-Methyl Derivatives of Ethosuximide (I) and Metabolite, KI 1480 (II)

Chemical Shift[a]		Coupling Patterns[b]
	I	
NCH_3	2.91	s
Ring CH_2	2.30 2.44	*AB*
Ethyl CH_2	1.74 1.68	pair q by 7.3 Hz
CH_3	1.26	s
Ethyl CH_3	0.87	t by 7.3 Hz
	II	
NCH_3	2.96	s
Ring CH	4.22	s
OH	2.59	bs
Ethyl CH_2	1.80 1.74	pair q by 7.3 Hz
CH_3	1.16	s
Ethyl CH_3	0.97	t by 7.3 Hz

[a] In parts per million downfield with respect to tetramethylsilane. [b] s = singlet, t = triplet, q = quartet, and b = broad.

The two major ions resulting from ring fission of the succinimide nucleus at *m/e* 86 and 71 corresponded to $C_5H_{10}O$ (calc., 86.0732; found, 86.0734) and C_4H_7O (calc., 71.0497; found, 71.0503). These ions could arise from either the *N*-methyl derivative of the ring-hydroxylated compound, II, or its positional isomer formed by hydroxylation at the C-2 methyl group. The latter structure, however, would be expected to yield a significant peak at *m/e* 141 corresponding to loss of CH_2O from M^+ *via* a McLafferty rearrangement similar to that proposed for $M^+ - C_2H_4$. Conclusive proof that KI 1480 represents the ring-hydroxylated derivative was obtained by preparative GLC isolation and subsequent proton NMR analysis.

The close correspondence of the proton spectra of the *N*-methyl-derivatives of ethosuximide (I) and KI 1480 (II) is summarized in Table I. Ring hydroxyl substitution in the methylated metabolite replaced the characteristic *AB* splitting pattern observed for the nonequivalent ring methylene protons in the methylated I with a singlet and a broad singlet at 4.33 and 2.59 ppm for the ring proton and the hydroxyl proton, respectively. Saturation of the sample solution with aqueous hydrochloric acid displaced the 2.59-ppm resonance to about 1.1 ppm, whereas all other resonances were unaffected. The quartet and triplet patterns are characteristic of the methylene and methyl protons of an ethyl group. The chain methylene protons are nonequivalent, as they are on a car-

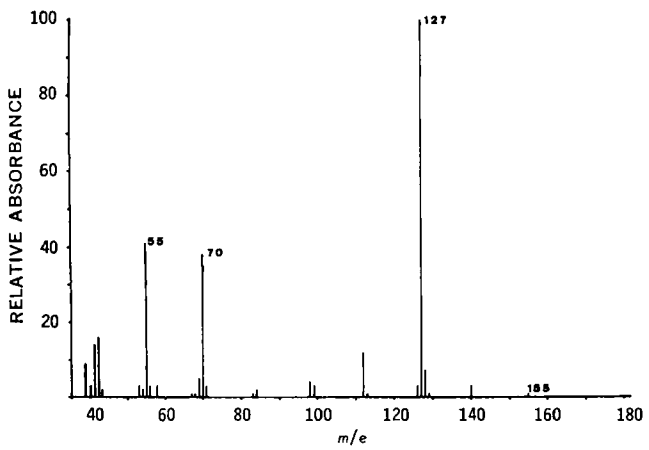


Figure 2—*Electron-impact mass spectrum of* N-*methylethosuximide (peak 1355, Fig. 1).*

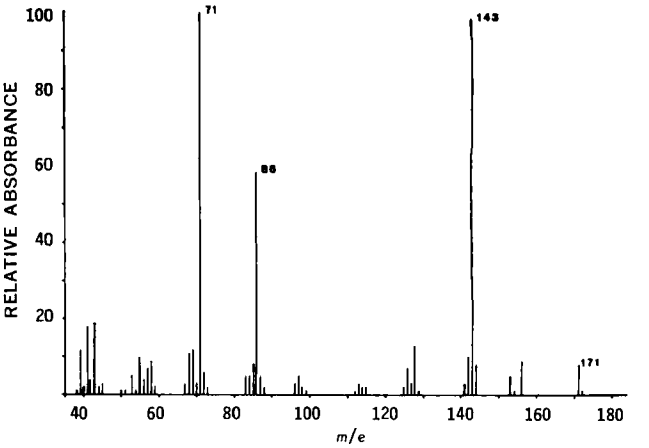


Figure 3—*Electron-impact mass spectrum of* N-*methyl-2-ethyl-2-methyl-3-hydroxysuccinimide (peak 1480, Fig. 1).*

bon bonded to an asymmetric carbon, and appear as a pair of overlapping quartets.

By combined GLC–mass spectrometry, KI 1595 and 1625 were also shown to represent *N*-methylmonohydroxyethosuximide derivatives (M^+ at m/e 171). The mass spectra of these compounds were virtually identical, indicating a stereoisomeric pair. The most intense peak in both spectra corresponded to loss of C_2H_4O from the molecular ion. In addition, the spectra were devoid of an m/e 143 peak corresponding to loss of C_2H_4 from M^+. On this basis it was concluded that these compounds represented the previously reported (1) stereoisomeric hydroxyethylethosuximides, 2-(1-hydroxyethyl)-2-methylsuccinimide.

Quantitative data were not obtained for the urinary excretion of II. However, its close similarity in structure to the previously reported hydroxyethyl metabolites should result in a nearly 1:1 ratio for isolation and subsequent GLC detection. The results in Fig. 1 thus indicate that II is present in nearly equal concentration as the previously identified hydroxyethyl derivatives. Similar results were obtained for 0–24-hr urine extracts obtained from two male volunteers receiving a single 500-mg dose of ethosuximide. The latter findings indicate that formation of II is not dependent on chronic ingestion of this drug.

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Synthesis and Antibacterial Properties of Substituted Decylbarbituric Acids

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Abstract □ A series of substituted decyloxybarbituric and decylthiobarbituric acids was prepared and evaluated for antibacterial activity. The acids were synthesized by condensing the appropriate disubstituted malonic ester with urea in a potassium *tert*-butoxide–dimethyl sulfoxide medium at room temperature. When tested against *Bacillus subtilis* and *Staphylococcus aureus*, the most active compound was 5-allyl-5-decylbarbituric acid. A number of other decyloxybarbituric acids showed some activity, but the decylthiobarbituric acids were completely inactive. All compounds were inactive against *Escherichia coli* and *Proteus vulgaris*.

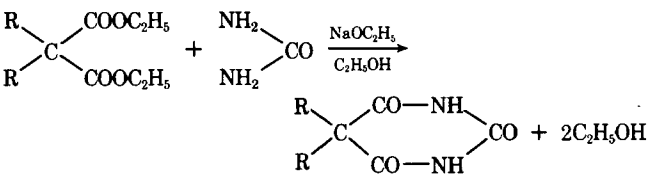
Keyphrases □ Barbituric acids, substituted decyl—synthesized and screened for antibacterial properties □ Decyloxybarbituric and decylthiobarbituric acids—synthesized and screened for antibacterial properties □ Antibacterial activity—substituted decyloxybarbituric and decylthiobarbituric acids

The most general method for the synthesis of barbituric acids has been the base-catalyzed condensation of a malonic ester with urea in refluxing ethanol (1) (Scheme I). Yields from this reaction are normally in the 50–75% range. The advantage of using a potassium *tert*-butoxide–dimethyl sulfoxide medium for this condensation was previously reported (2). This technique was used to synthesize a series of new decyl-substituted barbituric acids which were screened for antibacterial activity. Barbituric acid itself has been shown to inhibit the growth of *Escherichia coli* (3); sodium phenobarbital inhibits the growth of *E. coli*, several hemolytic streptococci, pneumococci, and *Haemophilus influenzae* (4); and several substituted quinolinium barbituric acid salts inhibit the growth of *Streptococcus* and *Staphylococcus* species (5).

RESULTS AND DISCUSSION

The disubstituted malonic esters (Table I) were prepared by condensing *n*-decyl bromide with the appropriate sodiomalonic ester in dimethylformamide according to a procedure modified from that of Zaugg *et al.* (6). The resulting diesters were then condensed with urea at room temperature in a potassium *tert*-butoxide–dimethyl sulfoxide medium to give the oxybarbituric and thiobarbituric acids (Table II). Of special note are the general improvement of the yields and the simplicity of the reaction workup as compared to the classical technique.

Compounds 6–12 were tested *in vitro* against four microorganisms (Table III): *Bacillus subtilis, Staphylococcus aureus, E. coli,* and *Proteus vulgaris*. The only activity found was against the Gram-positive organisms. All compounds were inactive against the Gram-negative organisms at 250 μg/ml, the highest concentration studied. The most active compound was the 5-allyl-5-decyl derivative, Compound 6. Substitution of sulfur for



Scheme I