Several compounds structurally related to IV*b* were examined for their ability to cross-react with the antiserum obtained, *i.e.*, their ability to displace radiolabeled IV*b* from antibody binding sites. Antibodies were unable to distinguish among IV*b*, I, and the structurally similar ergot alkaloids ergonovine and methylergonovine. Ergotamine also exhibited cross-reactivity, but at approximately 10-fold higher concentrations. The following indoles did not exhibit any ability to displace radiolabeled IV*b* from antibody binding sites at concentrations of at least 10 mcg./ml. of incubation mixture: tryptamine, *N*-methyltryptamine, 5-methoxydimethyltryptamine, 5-hydroxytryptophan, and 5-hydroxytryptamine (serotonin). No exhaustive study of cross-reactivity was completed; however, these results compare in general with those reported by Vunakis *et al.* (4). Judging from information supplied in a commercial kit[14], antiserum produced by other methods may exhibit somewhat greater specificity. One outstanding example appears to be that these authors report little cross-reactivity with I at concentration levels similar to IV*b*, while these two compounds appear essentially indistinguishable using our antiserum.

SUMMARY

A quick and convenient radioimmunoassay system was developed for the quantitative determination of lysergide (IV*b*) in drug products, human plasma, serum, or urine at levels as low as 1 ng. or for its qualitative detection at picogram levels. Liter quantities of antiserum were obtained since sheep were employed for the production of antibodies. An advantage of the method is in its utilization of commercially available tritiated IV*b*; however, the low specific activity of this material also places a limit upon the sensitivity. The method also has the advantage of not requiring a second antibody technique for the separation of free and bound IV*b* but is based upon adsorption of free IV*b* to dextran-coated charcoal. Current efforts are directed toward the acquisition of IV*b* of higher specific activity and the development of methods utilizing immobilized antibodies for efficiently concentrating IV*b* in biological samples so that the quantitative assay may be extended to the picogram levels required in the study of the absorption, distribution, metabolism, and excretion of IV*b*. However, even in its current state the method promises to be of considerable utility in the assay of illicit drug samples and in animal investigations where larger quantities of lysergide IV*b* are employed.

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▲ To whom inquiries should be directed.

[14] *Note added in proof*: Information cited in *Footnote 1* has now been reported: A. Taunton-Rigby, S. E. Sher, and P. R. Kelley, *Science*, **181**, 165(1973).

Identification of a Blood Metabolite of Methsuximide by GLC–Mass Spectrometry

I. A. MUNI▲, C. H. ALTSHULER, and J. C. NEICHERIL

Abstract □ The chemical structure of a prominent methsuximide metabolite was determined by combined GLC mass spectrometry. The experimental data indicate that the metabolite is an *N*-demethylated methsuximide. A simple GLC method is described for simultaneous estimation of methsuximide and its metabolite in blood samples.

Keyphrases □ Methsuximide—identification of *N*-demethylated metabolite in blood, man and rabbits, GLC–mass spectroscopy □ GLC–mass spectroscopy—identification, methsuximide *N*-demethylated metabolite in blood □ GLC—analysis, methsuximide and *N*-demethylated metabolite in blood

An important aspect in the treatment of patients with epilepsy is the determination of an effective and safe dosage schedule of anticonvulsant drugs. The estimation of these drugs in physiological fluids, particularly in blood, provides useful metabolic information for practitioners. Many drugs used are actively metabolized *in vivo* (1–3), and the identification and quantitation of these metabolites are of considerable clinical importance.

A general procedure for the determination of anticonvulsants in blood was recently described (4). When the method was applied to patients who had been receiving methsuximide (*N*,2-dimethyl-2-phenylsuccinimide), it became apparent that the drug had been rapidly metabolized both in humans and in rabbits and that the major metabolite had GLC characteristics different than the parent drug.

The purpose of this investigation was to determine the chemical structure of the methsuximide metabolite. The GLC properties of both the human and animal

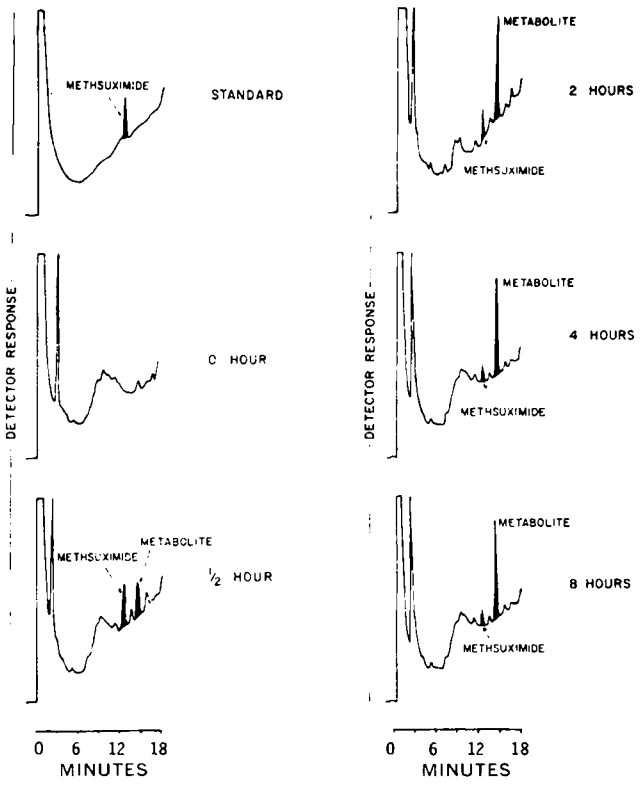


Figure 1—*Schematic diagram of methsuximide metabolism in rabbits (flame-ionization detection, 10% OV-17, 165–280°, 7.5°/min.).*

metabolite of methsuximide are similar. The present report describes its chemical characterization when separated from rabbit blood by GLC–mass spectrometry. A simple and rapid GLC method to determine the blood levels of methsuximide is also presented.

EXPERIMENTAL

Chemicals—Methsuximide[1], m.p. 50°, and methsuximide capsules[2] (300 mg.) were used. The solvents used were of analytical grade. *N,O*-Bis(trimethylsilyl)acetamide[3] was used for on-column trimethylsilylation.

Apparatus—An analytical gas chromatograph[4], equipped with a dual flame-ionization detector system, was used to monitor drug levels in blood samples. The separation was carried out on a 1.82-m. (6-ft.) long, U-shaped glass column packed with 10% OV-17 on Gas Chrom Q[5], 80–100 mesh.

The GLC–mass spectrometry[6] system interfaced to a data system[7] was used in the characterization of the methsuximide metabolite. A 0.61-m. (2-ft.) long, U-shaped glass column packed with 2% SE-30 on Gas Chrom Q[5], 80–100 mesh, was used for the separation of the metabolite. Monolinear temperature programming was used in both systems. The electron ionization energy was 70 ev. The ionization source temperature was maintained at 250°.

Procedure—*Drug Administration*—Several male rabbits, weighing 3.4–4.5 kg., were used. One methsuximide capsule (300 mg.) was administered orally to each animal, and the blood samples (about 4 ml.) were collected at intervals of 0.0, 0.5, 2.0, 4.0, and 8.0 hr.

Plasma Extraction—Two milliliters of plasma sample was acidified with 6 *N* HCl (50 μl.), and the drug as well as the metabolite was extracted with a mixture of 8 ml. of chloroform–isopropanol (9:1 v/v). The organic phase was filtered through phase-separating paper[8], and a 6-ml. sample of filtrate was concentrated to dryness at 35° under a gentle stream of nitrogen.

GLC Analysis—The dried samples were resuspended in 50 μl. of chloroform–isopropanol (1:1 v/v), and 2–5 μl. was injected into a gas chromatograph. Working standard curves of methsuximide were routinely established for drug quantitations.

GLC–Mass Spectrometry Studies—A chloroform–isopropanol extract (2 μl.) of the 4-hr. plasma sample was injected into a gas

Table I—Typical Recoveries of Methsuximide

Sample	Methsuximide Added, mcg.[a]	Methsuximide Found, mcg.[b]	Recovery, %
Human plasma	30	31	103
Human plasma	45	43	96
Human plasma	60	58	97
Human plasma	300	303	101
Rabbit plasma	30	29	97
Rabbit plasma	45	43	96
Rabbit plasma	60	61	102
Rabbit plasma	300	299	100

[a] Amounts of methsuximide in methanol (3 mg./ml.) added to 4 ml. of plasma sample. [b] Calculated to nearest whole number.

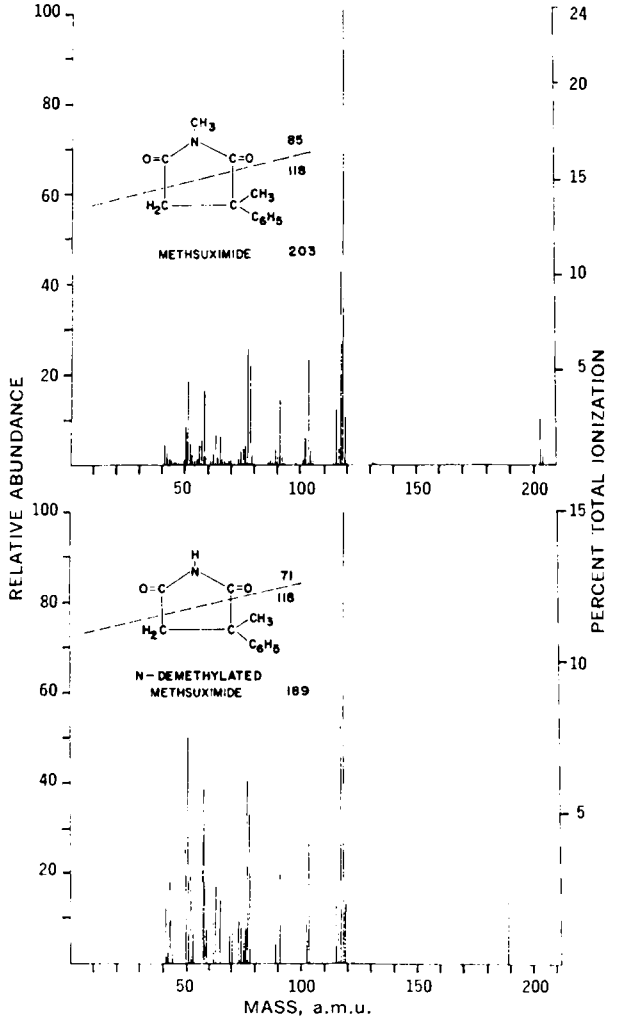
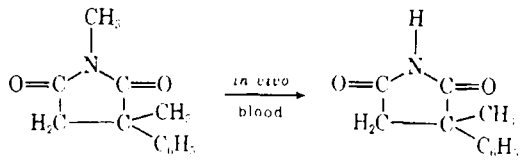


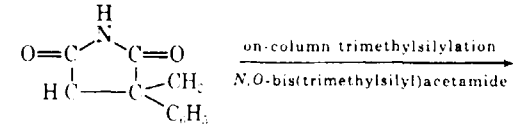
Figure 2—*Mass spectral fragmentation patterns of methsuximide and its metabolite.*

[1] Celontin, gift of Parke, Davis & Co., Detroit, Mich.
[2] Obtained from St. Joseph's Hospital pharmacy.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Barber-Colman model 5000.
[5] Applied Science Laboratories, State College, Pa.
[6] Hewlett-Packard gas chromatograph model 5780A and Hewlett-Packard mass spectrometer model 5930A.
[7] Hewlett-Packard data system model 5932A.
[8] Whatman.

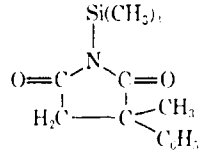


methsuximide (mol. wt. 203) *N*-demethylated methsuximide (mol. wt. 189)

Scheme I—Methsuximide metabolism



N-demethylated methsuximide (mol. wt. 189)



trimethylsilyl-*N*-demethylated methsuximide (mol. wt. 261)

Scheme II Trimethylsilylation of methsuximide metabolite

chromatograph, and the underivatized compounds were subjected to GLC–mass spectrometry. On-column trimethylsilylation was achieved by injecting a mixture of 2 μl. of *N,O*-bis(trimethylsilyl)-acetamide and 2 μl. of concentrated blood extract. A chloroform–isopropanol solution containing standard methsuximide was also subjected to GLC–mass spectrometry analysis.

RESULTS AND DISCUSSION

The relative blood levels of methsuximide and its metabolite at 0.5, 2.0, 4.0, and 8.0 hr. after oral administration of 300 mg. of methsuximide are illustrated in Fig. 1. Methsuximide metabolizes rapidly *in vivo* to a major metabolite, and after 0.5 hr. the concentration of this metabolite in blood is relatively much higher than that of unchanged methsuximide.

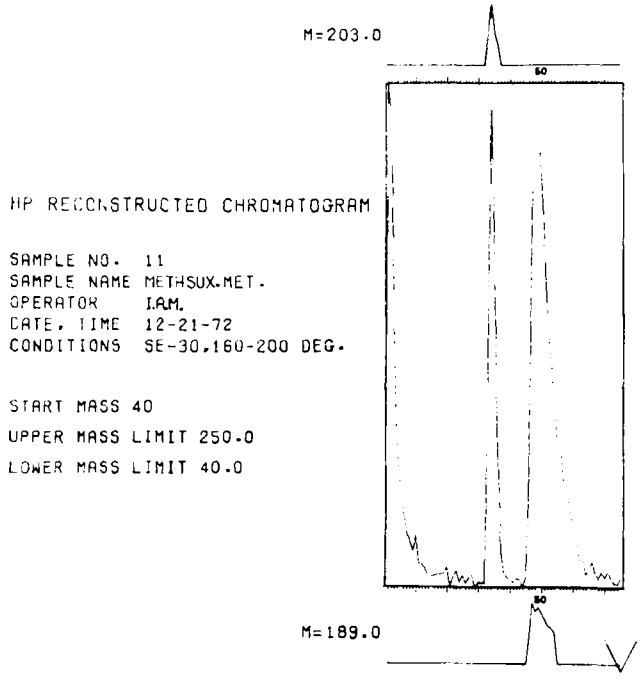


Figure 3—*Reconstructed chromatogram of plasma extract. M = 203 is a chromatographic peak for methsuximide, and M = 189 is a chromatographic peak for* N*-demethylated methsuximide.*

Table II—Relative Abundance of Several Fragment Ions of Methsuximide and Its Metabolite

Fragment Ion (*m/e*)	Relative Abundance, % Methsuximide	Relative Abundance, % Metabolite
203	10.22	– [a]
189	–	14.56
119	10.88	13.88
118	100.00	100.00
117	42.56	52.75
115	12.37	12.99
103	23.40	29.52
91	14.55	20.08
78	21.98	33.46
77	25.83	40.98
63	–	17.32
58	16.29	38.97
57	–	27.56
51	18.61	50.00
50	—	25.59
43	–	18.11
41	—	16.53

[a] Either no fragment ion was observed or the relative abundance (%) was less than 10%.

As shown in Fig. 1, methsuximide and its metabolite were successfully resolved on the OV-17 column; each compound produced a single GLC peak. Similar resolution was obtained using the SE-30 liquid phase. The flame-ionization detector gave a linear response for methsuximide, as well as its metabolite, under the experimental conditions used.

When added to plasma samples, the recoveries of methsuximide for both human and rabbit plasma were usually in the 96–103% range (Table I). Recoveries were comparable whether extractions were made immediately after adding methsuximide or after storing the plasma samples for 24–40 hr. at 5°.

As shown in Fig. 2, the mass spectra of methsuximide and its metabolite are simple, characteristic, and easy to interpret. The spectra were recorded from 0 to 650 a.m.u. In both spectra, the base peak (B) was observed at *m/e* 118 (Table II). Methsuximide and its metabolite were characterized by prominent clusters of fragment ions in the *m/e* 115–119, 73–78, and 50–58 regions. Methsuximide also showed significant peaks at *m/e* 203 (molecular ion, M), 103, and 91. The mass spectra of standard methsuximide and methsuximide extracted from blood samples were identical.

On-column trimethylsilylation resulted in a GLC peak shift for the methsuximide metabolite. The trimethylsilylated metabolite of methsuximide eluted earlier than the underivatized metabolite. On-column trimethylsilylation did not change the GLC retention time or the mass spectral characteristics of methsuximide. The mass spectrum of trimethylsilyl methsuximide metabolite showed fragment ions at *m/e* 261 (M), 246 (M − 15). 147 $[(CH_3)_2Si{=}O\ Si(CH_3)_3]$, 118, and 73 $[(CH_3)_3Si]$.

The GLC–mass spectrometry data indicate that the predominant blood metabolite of methsuximide is an *N*-demethylated methsuximide (Schemes I and II). *N*-Demethylation is the usual route of metabolism for several *N*-methylated anticonvulsant drugs (5, 6). Although demethylation ordinarily requires many hours for completion (5), metabolism of methsuximide seems to be relatively rapid in humans and animals.

Figure 3 is an illustration of the *XY* plot from the data system. The central portion of the figure is the total ion monitor output for all products with fragment ions between 40 and 250. The upper figure indicates that the first peak (scan numbers 30–40) had a fragment ion of 203, and the lower figure indicates that the second peak (scan numbers 48–58) had a fragment ion of 189.

SUMMARY

A relatively simple, rapid, and accurate GLC system is described. It was found to be useful in the monitoring of the physiological levels of methsuximide and its metabolite in one operation. The GLC–mass spectrometry data indicate that methsuximide is extensively metabolized *via N*-demethylation.

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▲ To whom inquiries should be directed.

Electronic Spectra and Electronic Structures of Aminoanthracenes

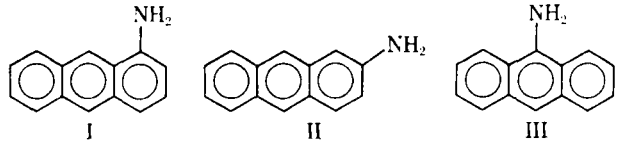
STEPHEN G. SCHULMAN▲, PETER J. KOVI, GEORGE TOROSIAN, HOWARD McVEIGH, and DORIS CARTER

Abstract □ The electronic absorption and fluorescence spectra of the cation derived from 9-aminoanthracene (9-anthrylamine), in water, are anomalous by comparison with those of the 1- and 2-anthrylammonium ions. The similarities of the spectra of the cation of 9-anthrylamine with those of anthrone and its cation lead to the conclusion that the former cation is not the 9-anthrylammonium ion but rather its tautomer, the protonated imine analogous to anthrone. In ethanol and in the solid state, measurable quantities of both the protonated imine and 9-anthrylammonium ions exist.

Keyphrases □ Aminoanthracenes—electronic spectra and electronic structures □ Absorption spectra, electronic, aminoanthracenes—determination □ Fluorescence spectra, electronic aminoanthracenes—determination □ Electronic spectra and electronic structures—determination, aminoanthracenes □ Anthrylamines—electronic spectra and electronic structures

The carcinogenicity of many polycyclic aromatic amines has been recognized since 2-naphthylamine was found to cause bladder cancers in workers in the dye industry (1, 2). Of the arylamines known to have carcinogenic properties, 1-aminoanthracene (I) and, especially, 2-aminoanthracene (2-anthrylamine) (II) are among the most virulent. However, to our knowledge, no carcinogenic properties have been reported for 9-aminoanthracene (III).

Of the functionally substituted aromatic molecules, the derivatives of anthracene have not been particularly well characterized, although many of them have been employed in the dye industry for many years. Recent studies have shown that, in particular, substitution in the 9-position of the anthracene ring often results in molecular species that are quite different in their chemical properties from other aromatic molecules (*e.g.*, benzene and naphthalene) substituted with the same functional groups. On the other hand, substitution in the 1- and 2-positions of the anthracene ring yields derivatives that are similar to the corresponding benzene and naphthalene derivatives. For example, in hydrocarbon solvents, the carboxyl groups of benzoic acid, 1- and 2-naphthoic acids, and 1- and 2-anthroic acids are coplanar and conjugated with the respective aromatic rings, while the carboxyl group of 9-anthroic acid (3) is perpendicular and unconjugated with the anthracene ring as a result of steric interference of the 9-carboxyl group with the peri hydrogen atoms in the 1- and 8-positions of the anthracene ring. Moreover, of the hydroxyanthracenes, 1- and 2-anthrols appear to be well-behaved phenolic molecules (4) while 9-anthrol exists predominately as its keto tautomer, anthrone, and only to a very slight extent as the phenolic 9-anthrol (5, 6).



I II III

Because of interests in the relationships between molecular electronic structure and biological (in this case carcinogenic) activity, the present investigation of the electronic structures of the isomeric aminoanthracenes by electronic absorption and fluorescence spectroscopy was undertaken.

EXPERIMENTAL

Reagents—The 1- and 2-aminoanthracenes[1], anthracene, and 9-aminoanthracene hydrochloride[2] were each recrystallized several times from absolute ethanol.

Analytical reagent grade sulfuric acid[3] was diluted with distilled, deionized water to prepare the solutions used to study the Hammett acidity region. Solutions in the pH range were citrate and phosphate buffers and sodium hydroxide solutions in distilled, deionized water.

Each sulfuric acid or buffer solution in a 10-ml. volumetric flask was injected with 100 μl. of a 1×10^{-2} *M* stock solution of the appropriate aminoacridine in absolute ethanol immediately prior to

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] K&K Chemical Co., Plainview, N. Y.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.