Compound II was chosen on the basis of its structural similarity to I. Due to its similar pKa (7.90) (25), II can be added directly to the plasma sample. Compound II also is easily and quantitatively permethylated with the method described. The derivative obtained is well extracted from the reaction mixture before GLC and yields excellent chromatographic characteristics on the 3% OV-17 column system. The chromatograms (Figs. 1 and 2) showed no major interfering peaks eluting in the regions corresponding to I and II.

The use of the thermionic nitrogen detector (26) provides higher sensitivity and better specificity for nitrogen-containing compounds. A linear relationship between peak height ratios of I to II and plasma concentrations of I was demonstrated in the 0–1-μg/ml range (regression line: $y = 1.02x - 0.01$, $r = 0.996$). A detection limit of 50 ng of I/ml was obtained by injecting 1.0 μl of the 50 μl of ethyl acetate from the reconstituted sample residues, using 1.0 ml of plasma for analysis.

The within-run precision of the overall procedure was determined by analyzing 12 plasma samples spiked with 300 ng of I/ml. A standard deviation and a coefficient of variation of 12 ng/ml and 4.0%, respectively, were found. Analysis of 20 plasma samples, also spiked with 300 ng of I/ml, over 3 weeks yielded some indication of the long-term precision, namely, 18 ng/ml (*SD*) and 6.2% (*CV*). These data on replicate analysis and long-term precision are presented in Table I and are acceptable for an analysis at the submicrogram level.

The described procedure is an example of the superiority of the thermionic nitrogen detector because of its selectivity for nitrogen-containing compounds. Although interference from other endogenous substances was expected, blank plasma samples and plasma samples spiked with I at the submicrogram level were taken through the procedure and gave no significant peaks that might interfere. Besides this increased selectivity, sensitivity is increased approximately 20 times compared to the flame-ionization detector.

The applicability of this method for plasma samples collected from patients treated with I was not demonstrated because I is not used as a chemotherapeutic agent to treat cancer cases in our medical environment. Since I occurs as a metabolite in humans treated with the antineoplastic agent fluorouracil, development of the described procedure is important. Therefore, we propose to extend our method for the assay of I in urine and tumorous tissue.

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ACKNOWLEDGMENTS

Supported by the National Medical Research Foundation (F.G.W.O.) through Grants 20452 and 3.004.76.

The authors are indebted to Produits Roche N.V., Brussels, Belgium, for their gift of I.

GLC Determination of Pemoline in Biological Fluids

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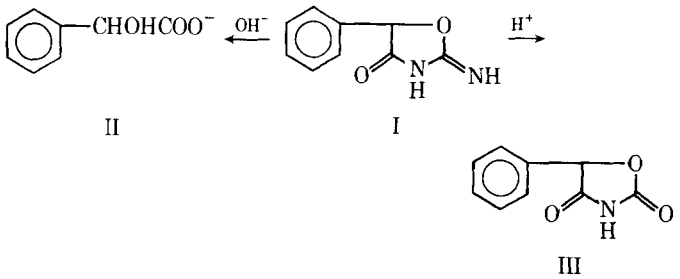
Received October 26, 1976, from the *Department of Pharmacy, University of Antwerp, Antwerp, Belgium.* Accepted for publication June 22, 1977.

Abstract □ A specific GLC method for the determination of microgram quantities of the central stimulant pemoline in biological fluids is described. Extraction problems due to the low solubility of pemoline are avoided by acid hydrolysis of the drug to 5-phenyl-2,4-oxazolidinedione, which then can be easily isolated by two-phase extraction with dichloromethane, ether, or chloroform. Amide–imide tautomerism enables a cleanup of the extract. Quantitative determination at the microgram level is done on a methylated fraction of the dichloromethane extract by GLC using a suitable internal standard. For supporting evidence of the GLC method's specificity, the compound is also identified by examining an aliquot of the final dichloromethane extract by TLC.

Keyphrases □ Pemoline—GLC analysis in biological fluids □ GLC—analysis, pemoline in biological fluids □ Stimulants, central—pemoline, GLC analysis in biological fluids

The synthesis of a series of oxazolidinones was reported (1), and later (2) the central stimulant activity of pemoline (2-imino-5-phenyl-4-oxazolidinone) was noted. Its pharmacology also was reviewed (3, 4).



Scheme I

The detection methods for pemoline (I) in biological fluids consist of its conversion to mandelic acid (II) (Scheme I) and subsequent spectrophotometric or GLC determination (5, 6). These methods are not specific because mandelic acid also is produced by metabolism of styrene and other drugs. A major drawback of the spectrophotometric determination is a definite lack of sensitivity. Pemoline is practically insoluble in water, ether, and acetone but is soluble in hot alcohol and dimethyl sulfoxide. Because of its low solubility, extraction from biological fluids is the major problem.

This report concerns the development of a specific extraction procedure and the subsequent determination by TLC and GLC of pemoline in urine samples of athletes suspected of drug abuse.

EXPERIMENTAL

Sample Collection—Twenty-four-hour urine outputs from a male volunteer were collected following a therapeutic pemoline dose (40 mg po). The samples were stored at −22° until analysis. A plasma sample was taken 4 hr after pemoline administration and immediately analyzed.

Pemoline Hydrolysis—A saturated aqueous solution of pemoline[1] was hydrolyzed with an equal volume of 3.6 *N* H_2SO_4 in a boiling water bath for 1 hr. After cooling, the solution was extracted twice with dichloromethane; the combined dichloromethane layers were evaporated to dryness *in vacuo* at 40°. The residue was taken up in hot methanol and quickly filtered, and the methanol was evaporated, yielding a white crystalline powder, mp 107°.

This powder was fairly soluble in methanol, ethanol, ether, and dichloromethane and sparingly soluble in hexane, benzene, toluene, and petroleum ether. It formed water-soluble salts with sodium hydroxide. The structure of the hydrolysis product, 5-phenyl-2,4-oxazolidinedione (III) (Scheme I), was confirmed by IR (potassium bromide), NMR (deuterochloroform), and mass spectral data.

Assay for Pemoline in Urine—Urine (10 ml) was acidified with 1 ml of 36 *N* H_2SO_4 and hydrolyzed in a boiling water bath for 1 hr. After cooling, an amount of phosphotungstic acid, sufficient to discolor the urine and precipitate interfering materials, was added. After centrifugation, the supernate was decanted and extracted.

Assay for Pemoline in Serum or Plasma—Serum or plasma (10 ml) was thoroughly mixed with 10 ml of 10% trichloroacetic acid. After centrifugation, the protein-free supernate was hydrolyzed with 0.5 ml of 36 *N* H_2SO_4 in a boiling water bath for 1 hr and subsequently extracted.

Extraction—The hydrolysis product, III, was extracted with three 20-ml portions of dry dichloromethane, and the combined organic fractions were extracted twice with 5 ml of 0.5 *N* NaOH. The aqueous layers were acidified with 10% H_2SO_4 and reextracted with three 20-ml portions of dichloromethane. The combined dichloromethane layers were evaporated *in vacuo* at 40° to near dryness and derivatized.

Derivatization—A known amount of the internal standard, allobarbital, was added to the remaining dichloromethane solution (about 0.5 ml), which was then treated dropwise with a solution of diazomethane in dry dichloromethane (7) until a yellow color persisted for 1 min. The solution was washed with three 5-ml portions of 0.5 *N* NaOH, dried over anhydrous sodium sulfate, transferred to a 15-ml glass-stoppered silanized vial, and evaporated with a nitrogen stream at room temperature. The derivatization product was 3-methyl-5-phenyl-2,4-oxazolidinedione (IV) according to IR, NMR (deuterochloroform), and mass spectral data.

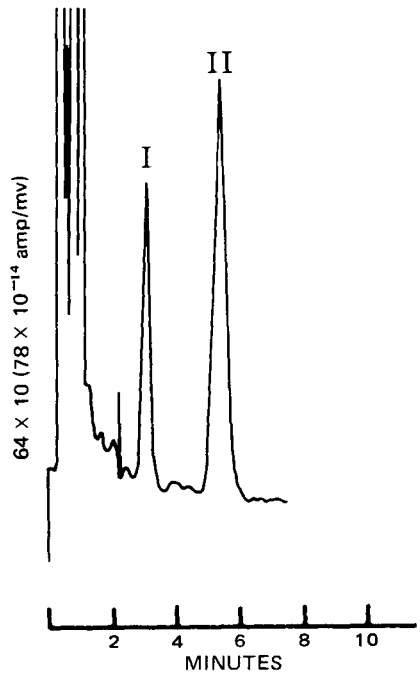


Figure 1—*Gas chromatogram of an extract of 20 ml of urine taken 14 hr after administration of 40 mg of pemoline. Peaks I and II are due to methylated allobarbital and a 2-μg injection of IV, respectively.*

TLC—The extract was dissolved in 100 μl of ethanol, and 20 μl of this solution was spotted on a precoated silica gel 60 F_{254} plate[2], together with known amounts of IV. The plate was run in a developing chamber previously saturated with chloroform–hexane (50:50). After development, the plate was air dried. Then the spots were located with iodoplatinate (Reagent A) or with a freshly prepared solution of 0.5% fast blue salt B[3] in water followed by 1 *N* NaOH (Reagent B).

In the presence of pemoline, a red spot with R_f 0.30 appeared with Reagent A (detection limit = 5 μg) and a yellow spot appeared with Reagent B (detection limit = 3 μg).

GLC—A gas chromatograph[4] equipped with a dual flame-ionization detector was used. The column consisted of 3% phenylmethyl dimethyl silicone[5] coated on 100–120-mesh diatomaceous earth[6] packed in a 2-m × 2.5-mm i.d. silanized Pyrex column. Analyses were carried out isothermally at an oven temperature of 180°; injector and detector temperatures were maintained at 200°. Nitrogen was used as carrier gas at 30 ml/min (bubble flowmeter). All injections were made on-column with a 10-μl syringe[7], and about 1 μl of the extract with allobarbital as the internal standard was injected (Fig. 1). Peak areas were measured using an electronic calculator[8].

RESULTS AND DISCUSSION

The extraction of pemoline from biological fluids presented the major drawback. Acid hydrolysis of pemoline to III, which is very soluble in organic solvents, was the most obvious method. Furthermore, because III is a weak acid, it could be extracted back into alkali for cleanup (amide–imide tautomerism) (8). Interfering material, mainly due to the acid hydrolysis of urine, could be removed almost completely by phosphotungstic acid without a noticeable loss of pemoline.

The use of anhydrous sodium sulfate, even analytical grade, was avoided because it introduced many impurities that interfered in the GLC

[1] Aldrich Chemicals.

[2] Merck.
[3] Fluka AG, Buchs, Switzerland.
[4] Hewlett-Packard 5170 A.
[5] OV-7.
[6] Gas Chrom Q, Applied Science Laboratories.
[7] Hamilton 701 N, Whittier, Calif.
[8] Hewlett-Packard 3380 A.

Table I—Recoveries of Pemoline Added to Human Urine Samples

Pemoline Added to 20 ml of Human Urine, μg	Amount Recovered, μg	Recovery, %
25.5	25.1	98.4
51	49.9	97.8
102	100	98.0
204	203.5	99.8
408	403.5	98.9
	Mean value ± *SD*	98.6 ± 0.80

analysis. Therefore, organic layers were dried by cooling for 15 min; remaining water droplets were removed with filter paper.

Pemoline could not be chromatographed even on nonpolar liquid phases. The product of acid hydrolysis, III, is more volatile, but peaks tend to tail on columns of various polarities. When 40–60-mesh polytetrafluoroethylene polymer[9] was used as support material, some improvement in peak symmetry was observed, but the columns were of low efficiency. Methylation with diazomethane to IV yielded a very volatile compound that demonstrated excellent peak symmetry on fairly nonpolar liquid phases.

Since the *N*-methyl derivative is insoluble in alkali, a final cleanup was possible by extracting the organic layer with sodium hydroxide.

Because of its similarity with the hydrolysis product of pemoline in chemical behavior, allobarbital was chosen as an internal standard. Both drugs are weak acids and can be reextracted into alkali, so allobarbital can be added immediately after hydrolysis. Furthermore, both products form *N*-methyl derivatives with diazomethane, so the final cleanup with sodium hydroxide solution gives no loss.

Recovery studies from urine and plasma were carried out by adding the internal standard before derivatization; in all other experiments, the internal standard was added immediately after hydrolysis. Addition of pemoline to five 20-ml urine samples in the range anticipated for therapeutic levels gave a recovery of 98.6 ± 0.80%. All determinations were carried out in duplicate, the lowest recovery from a single determination being 97.8% (Table I). Although recoveries were high, extreme care had to be taken during evaporation since IV and the methylated allobarbital are extremely volatile.

[9] Chromosorb T, Applied Science Laboratories.

Table II—Recoveries of 10 μg of Pemoline Added to Human Plasma Samples

Amount Recovered[a], μg	Recovery, %
10.20	101.5
9.70	96.3
9.80	97.4
9.90	98.2
9.90	98.6
Mean value ± *SD*	98.4 ± 1.94

[a] Values of the recovered IV without correction to I.

Addition of pemoline to human plasma yielded a recovery of 98.4 ± 1.94% (Table II). Since the normal range of plasma concentrations is 1.0 μg/ml, 10-ml samples were required when using a flame-ionization detector. Efficiency, however, could be improved markedly by using a nitrogen detector[10]. Although pemoline gave no increased nitrogen signal, the extreme stability and high noise-to-signal ratio of the detector allowed quantitative estimation of 20 ng of pemoline, which is sensitive enough to require 1 ml of plasma. Oral intake of 40 mg of pemoline, a therapeutic dose, gives a plasma concentration of 0.8 μg/ml at about 4 hr. Determination in urine is still possible 48 hr after ingestion due to the long elimination time of pemoline.

A complete elimination and metabolism pattern of pemoline is currently being investigated.

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Synthesis of 4,5-Dimethoxykynuramine and Its *In Vivo* Conversion to 6,7-Dimethoxy-4-quinolinol

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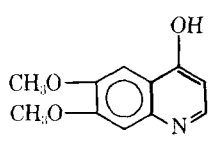
Received November 8, 1976, from the *Research and Development Department, Norwich-Eaton Pharmaceuticals Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication June 14, 1977. *Present address: Wyeth Laboratories, West Chester, PA 19380.

Abstract □ 4,5-Dimethoxykynuramine was synthesized in a three-step sequence originating with veratrole. Indirect evidence indicates that the drug was converted *in vivo* to the hypotensive agent 6,7-dimethoxy-4-quinolinol by the action of monoamine oxidase.

Keyphrases □ 4,5-Dimethoxykynuramine—synthesized, *in vivo* conversion to 6,7-dimethoxy-4-quinolinol □ 6,7-Dimethoxy-4-quinolinol—formed *in vivo* from 4,5-dimethoxykynuramine □ Antihypertensive agents—6,7-dimethoxy-4-quinolinol, formed *in vivo* from 4,5-dimethoxykynuramine

The hypotensive and antihypertensive activities of 6,7-dimethoxy-4-quinolinol (I) were demonstrated previously (1).



I

Since it had been shown that the *in vitro* incubation of kynuramine (II) with monoamine oxidase gave 4-quinolinol (III) (Scheme I) (2), the possibility of the conversion of 4,5-dimethoxykynuramine (IV) to the aldehyde V and then the quinolinol I (Scheme II) was considered. Should this transformation occur *in vivo*, IV might be considered a useful prodrug for I.