Journal of Chromatography, 134 (1977) 415–421

CHROM. 9759

COMBINED GAS–LIQUID CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF PEMOLINE IN BIOLOGICAL SAMPLES

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(Received October 4th, 1976)

SUMMARY

A description is given of a gas–liquid chromatographic (GLC) method for the detection and determination of pemoline in biological samples. On treatment with hydrochloric acid, pemoline is converted into 5-phenyl-2,4-dioxooxazolidine, an acidic compound, which can be easily extracted with dichloromethane and determined by GLC. A combined GLC–mass spectrometric method is described.

INTRODUCTION

The central nervous system activity of pemoline (5-phenyl-2-imino-4-oxo-oxazolidine)*, has been known since 1956[1]. The level of the stimulating effect of this compound is situated between that of amphetamine and caffeine. More recently, the product has been found to be useful in treating Parkinson's disease[2,3]. Neither its metabolism nor urinary excretion has yet been established. It is not known whether or not pemoline is excreted as such or in the form of metal chelates, which are easily formed with divalent ions such as Ca^{2+} and Mg^{2+}. Some of these chelates are also used as drugs**,[4]. Pemoline is also known as a drug of abuse.

Thin-layer chromatography (TLC) has been used for the detection of pemoline in urine[5] but is claimed to lack sensitivity. Recently, high-performance liquid chromatography (HPLC) has been applied succesfully to urine samples[6]. Gas–liquid chromatography (GLC) has been used for the determination of pemoline in pharmaceutical products, the active compound first being converted into benzaldehyde by oxidative degradation[7]. It is clear that this method lacks specificity when applied to biological samples.

This paper describes a simple GLC method for the detection of pemoline in urine and blood, following acid hydrolysis to 5-phenyl-2,4-dioxooxazolidine and/or methylation to the corresponding N-methyl derivative. The reactions are shown in Fig. 1.

* Trade-names: Phenoxine, Sofro, Stimul, Anform.
** Trade-names: Cylert, Ecylert.

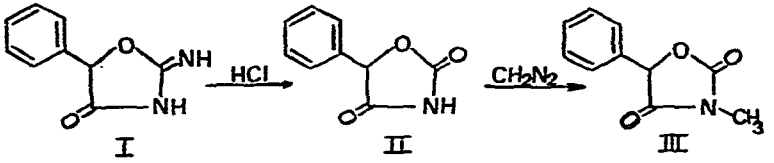

Fig. 1. Transformation of pemoline (I) into 5-phenyl-2,4-dioxooxazolidine (II) and subsequent methylation to 5-phenyl-2,4-dioxo-N-methyloxazolidine (III).

EXPERIMENTAL

Reagents

All solvents were of analytical-reagent grade. Pemoline was kindly supplied by Certa (Brussels, Belgium).

5-Phenyl-2,4-dioxooxazolidine was prepared according to Traube and Ascher[8] by heating for 6 min a suspension of 500 mg of pemoline in 100 ml of 1 *N* hydrochloric acid. After cooling, the mixture was extracted twice with dichloromethane, dried over anhydrous sodium sulphate and evaporated to dryness. The crude residue was recrystallized from water to give feathery plates of m.p. 109° in a yield of 98%.

5-Phenyl-2,4-dioxo-N-methyloxazolidine was prepared by adding an excess of an ethereal solution of diazomethane to 200 mg of 5-phenyl-2,4-dioxooxazolidine in diethyl ether. The mixture was allowed to stand in the cold for 10 min and, after evaporation of the diethyl ether, the residue was recrystallised from the same solvent. White plates of m.p. 114° were obtained in a yield of 97%.

The identities of the above two compounds were confirmed by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The NMR spectra were recorded on a Hitachi Perkin-Elmer R24 apparatus with tetramethylsilane as the internal standard for organic solvents. The products were dissolved in deuterated acetonitrile. The spectrum of 5-phenyl-2,4-dioxooxazolidine exhibited only two singlets, one at $\delta = 5.78$ (integrating for one benzylic proton) and the other at $\delta = 7.43$ (integrating for five phenyl protons); no signal for the NH proton was obtained. 5-Phenyl-2,4-dioxo-N-methyloxazolidine exhibited three singlets at $\delta = 3.0$ (integrating for three N-methyl protons), $\delta = 5.82$ (integrating for one benzylic proton) and $\delta = 7.43$ (integrating for five phenyl protons). The mass spectra were recorded on a single-focusing AEI-MS 12 mass spectrometer, operated at an accelerating voltage of 8 kV, trap current 100 μA and ionization energy 70 eV. The direct inlet technique was used, with a source temperature of 120–130°.

Standard solutions of 5-phenyl-2,4-dioxooxazolidine and of 5-phenyl-2,4-dioxo-N-methyloxazolidine were prepared for use each time.

Preparation of biological extracts

Concentrated hydrochloric acid (1 ml) was added to 10-ml samples of urine obtained from volunteers who had taken 20 mg of pemoline orally. The mixture was heated on a steam-bath for 4 min, then the cooled hydrolyzate was adjusted to pH 9 and extracted twice with 20 ml of dichloromethane. The organic layers were discarded and the aqueous solution was re-adjusted to pH 2 with concentrated hydrochloric acid and re-extracted with two 25-ml volumes of dichloromethane. The collected

organic extracts were dried over anhydrous sodium sulphate and evaporated to dryness on steam-bath under a stream of nitrogen. The residue was dissolved in 100 μl of dichloromethane containing 50 μg of methaqualone as an internal standard. Aliquots of 2 μl were used for injections into the gas chromatograph.

Blood samples (2 ml) were hydrolyzed with 2 ml of 2 *N* hydrochloric acid for 4 min and the mixture was treated as described above. The final acidic extracts were cleaned up on a Florisil column according to the procedure of Daenens and Bruneel[9]. After elution with 100 ml of dichloromethane, the purified extracts were evaporated to dryness and the residue was dissolved in 5 μl of dichloromethane containing 0.2 μg of internal standard. Aliquots of 3 μl were used for injection.

Urine extracts or purified blood extracts were methylated with excess of ethereal diazomethane. The mixture was allowed to stand in the cold for 10 min and the solvent was evaporated. The residue was dissolved in 100 μl of dichloromethane containing 50 μg of amobarbital as an internal standard (with urine samples) or in 5 μl of dichloromethane containing 0.2 μg of amobarbital (with blood samples).

Gas–liquid chromatographic conditions

A Varian Model 1400 gas chromatograph was used with a flame-ionization detector and 1.8 m × 2 mm I.D. glass columns packed with either 2% OV-17 on 100–120-mesh Gas-Chrom Q (column A) or 2% OV-225 on Chromosorb W-HP (column B). The column temperature was 180° (A) or 190° (B), the injector and detector temperature 220° and the gas flow-rates were carrier gas (nitrogen) 30, hydrogen 30 and air 300 ml/min. When methylated extracts were examined, the same operating conditions were used except that the oven temperature was 160° (column A).

The retention times are given in Table I. Quantitation was effected by adding a known mass of internal standard (methaqualone or amobarbital) to a range of standard solutions of the hydrolysis product or its methylated derivative. A calibration graph was constructed from the areas of the peaks after GLC separation. The peak areas were calculated with a Kipp digital integrator and linear calibration graphs were obtained from the peak-area ratios at several sample concentrations.

TABLE I

RETENTION TIMES (min) OF COMPOUNDS AND INTERNAL STANDARDS

Compound	*OV-17 (2%)*		*OV-225 (2%), 190°*
	160°	*180°*	
Amobarbital	12.8	6.2	12.5
Methaqualone	—	28.0	44
5-Phenyl-2,4-dioxooxazolidine	—	12.8	30
5-Phenyl-2,4-dioxo-N-methyloxazolidine	16	4.6	7

Gas–liquid chromatography combined with mass spectrometry

The GLC conditions were the same as described above. Helium was used as the carrier gas (flow-rate 50 ml/min). A membrane separator (Varian, Type 5620) allowed the eluates to flow into the ion source. The temperature of the separator and ion source was kept 30–40° above the column temperature.

Thin-layer chromatography

Standard techniques of ascending TLC were used. Runs of 16 cm were made on silica gel G, Type 60 (Merck, Darmstadt, G.F.R., Cat. No. 7731). Different solvent systems were used, the best results being obtained with chloroform–acetone (9:1) and cyclohexane–methanol–dioxan–diethylamine (30:10:10:1). The spots were revealed by means of short-wave UV light or by spraying with mercury(I) nitrate solution. In Table II R_F values and detection results are presented for 5-phenyl-2,4-dioxooxazolidine, 5-phenyl-2,4-dioxo-N-methyloxazolidine and some reference compounds.

TABLE II

R_F VALUES OF COMPOUNDS AND REFERENCE MATERIALS

Compound	*Chloroform–acetone (9:1)*	*Cyclohexane–methanol–dioxan–diethylamine (30:10:10:1)*	*UV light*	*Mercury(I) nitrate spray*
Amobarbital	0.31	0.47	–	+
Caffeine	0.12	0.39	+	–
5-Phenyl-2,4-dioxo-oxazolidine	0.30	0.20	+	+
5-Phenyl-2,4-dioxo-N-methyloxazolidine	0.49	0.50	+	+
Pemoline	0.02	0.02	+	+

RESULTS AND DISCUSSION

Previous experiments have shown that the reaction time needed for complete hydrolysis of micro-amounts of pemoline (1–100 μg) in urine and blood was 4 min. 5-Phenyl-2,4-dioxooxazolidine, having acidic properties, can be completely extracted with dichloromethane or diethyl ether from aqueous medium below pH 3.

The sensitivity for urine extracts is about 20 ng/ml, although for blood extracts with no prior purification the levels are at the limit of detection when therapeutic doses are taken. This effect is due partially to the presence of extraneous materials in the impure extracts. A clean-up on a Florisil column[9] or scraping a definite zone from a thin-layer plate increases the sensitivity. Methylation also enhances the sensitivity as small amounts of 5-phenyl-2,4-dioxooxazolidine may not be seen owing to tailing, even when polar phases are used. The peak of the N-methyl derivative, however, becomes very sharp and symmetrical. For blood, the sensitivity is about 40 ng/ml when the extracts are purified on Florisil and examined as the N-methyl derivative.

Typical gas chromatograms of urine extracts before and after ingestion of pemoline and prepared as described above are shown in Fig. 2. The urine samples examined were collected 2–4 h after ingestion of 20 mg of pemoline. Concentrations from 1 to 4 μg per millilitre of urine were observed. Fig. 3 shows a typical gas chromatogram obtained from blood collected 1 h after ingestion of 20 mg of pemoline. 5-Phenyl-2,4-dioxo-N-methyloxazolidine was present in a concentration of about 0.1 μg/ml.

The identities of the GLC peaks were confirmed by mass spectrometry. The spectra were identical with the spectra of 5-phenyl-2,4-dioxooxazolidine and/or 5-phenyl-2,4-dioxo-N-methyloxazolidine injected under the same conditions. The mass spectra, including the most important fragmentations, are shown in Figs. 4 and 5.

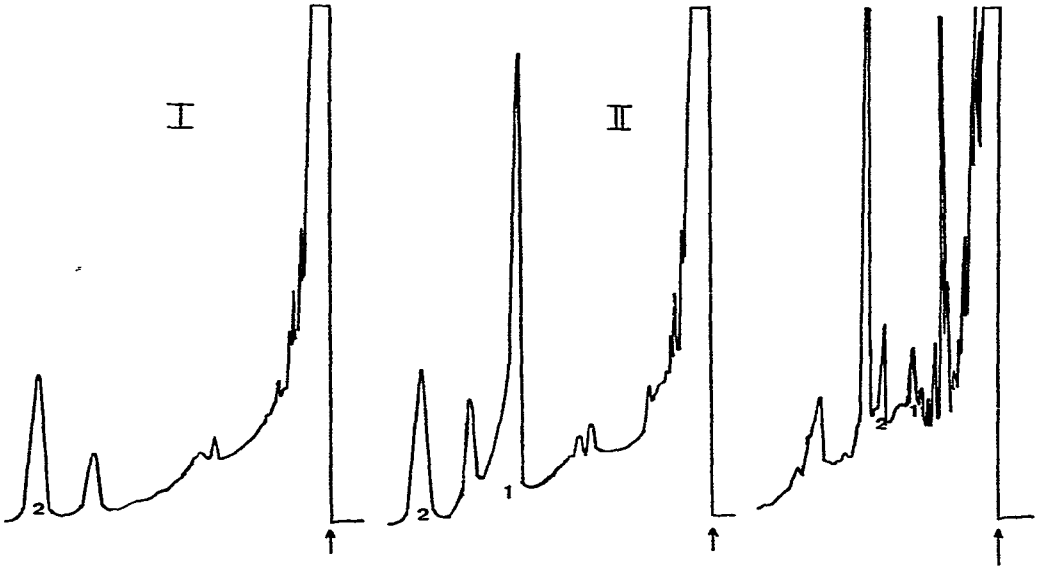

Fig. 2. Typical gas chromatograms of urine extracts (I) before and (II) 2 h after ingestion of 20 mg of pemoline. Column: 2% OV-225 on Chromosorb W HP. Peaks: 1 = 5-phenyl-2,4-dioxooxazolidine; 2 = internal standard (methaqualone).

Fig. 3. Typical gas chromatogram of blood extract, previously purified on Florisil and methylated. Column: 2% OV-17 on Gas-Chrom Q. Peaks: 1 = 5-phenyl-2,4-dioxo-N-methyloxazolidine; 2 = internal standard (amobarbital).

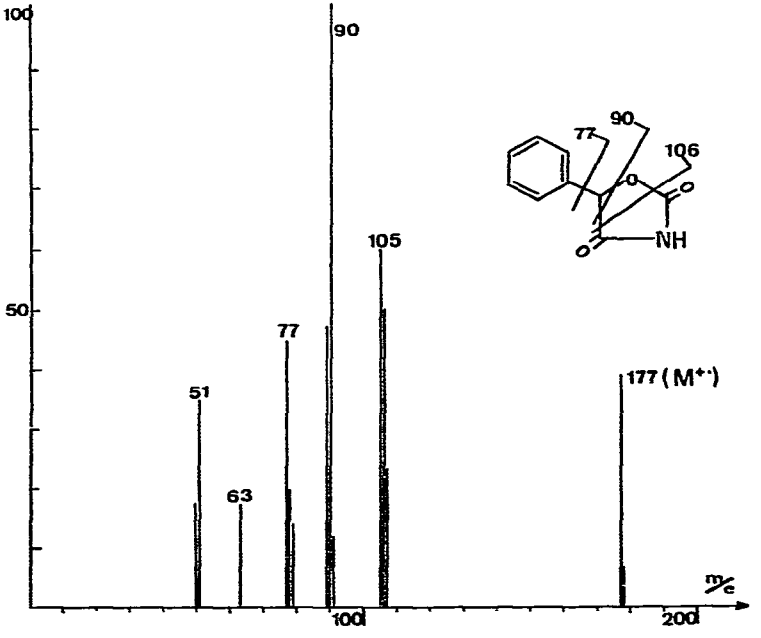

Fig. 4. Mass spectrum of 5-phenyl-2,4-dioxooxazolidine.

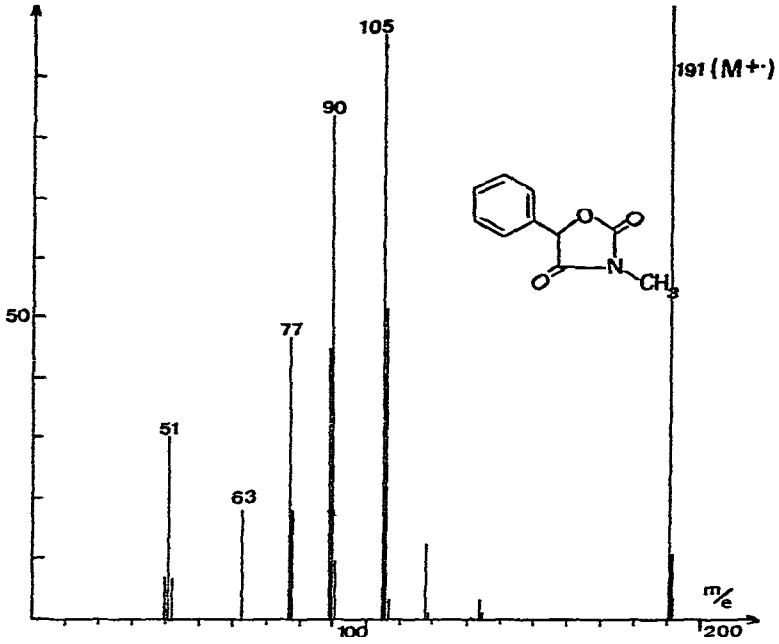

Fig. 5. Mass spectrum of 5-phenyl-2,4-dioxo-N-methyloxazolidine.

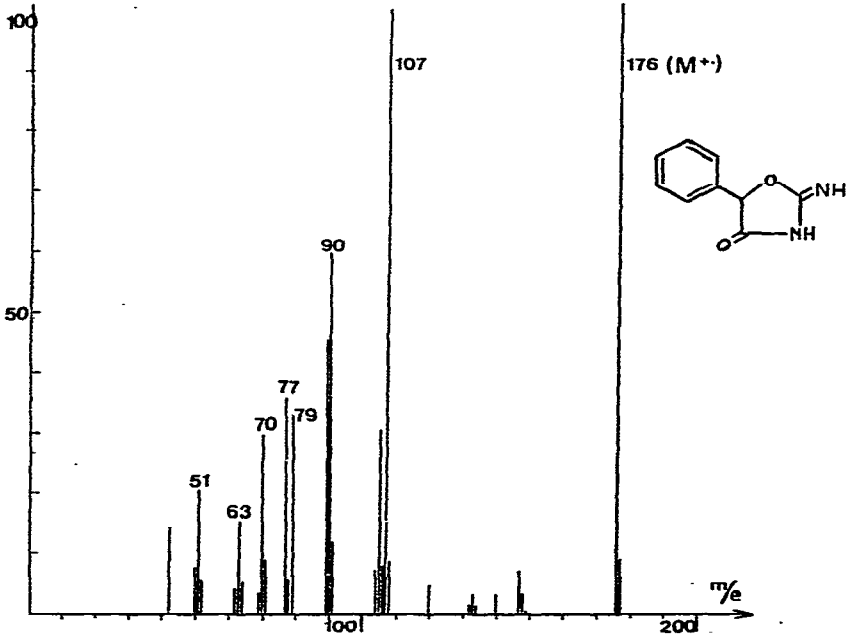

Fig. 6. Mass spectrum of 5-phenyl-2-imino-4-oxooxazolidine (pemoline).

Fig. 6 shows the mass spectrum of pemoline itself. The fragmentations are very similar. Instead of a molecular ion at *m/e* 176 for pemoline a molecular ion peak at *m/e* 177 for 5-phenyl-2,4-dioxooxazolidine was seen, the =NH group being replaced by an oxygen atom. The corresponding N-methyl derivative showed a molecular ion (100% peak) at *m/e* 191.

Although alkaline hydrolysis to mandelic acid[8] and its subsequent GLC detection could be considered as an alternative method for the detection of pemoline in biological fluids, acid hydrolysis is still to be preferred. The structural analogy of 5-phenyl-2,4-dioxooxazolidine with pemoline makes this product ideal for the detection of the active compound. The method permits the quantitative determination of pemoline in blood and urine with high specificity and sensitivity.

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