Spectrophotometric Determination of Pemoline and Mandelic Acid in Biological Fluids

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Abstract □ A rapid, simple spectrophotometric method which can be used to determine microgram quantities of pemoline and mandelic acid in biological fluids is described. To determine pemoline in serum the compound is hydrolyzed to mandelic acid and then oxidized to benzaldehyde with ceric sulfate. The benzaldehyde is extracted with hexane and its characteristic UV absorption is determined at 241 mμ. To determine urine levels, an additional purification step, involving the use of a cation-exchange resin, is required. The procedures described for the pemoline assay in serum and urine are also applicable for the determination of mandelic acid. Only data on the above compounds are presented in this paper but the method should be capable of determining many other compounds which can be converted to benzaldehyde.

Keyphrases □ Pemoline, biological fluids—analysis □ Mandelic acid—pemoline hydrolysis product □ Ion-exchange column—separation □ UV spectrophotometry—analysis

Recently, it has been reported that pemoline[1] and magnesium hydroxide (PMH),[2] a mild stimulant, enhances learning and memory (1). With such a drug it is highly desirable to be able to determine blood and urine levels so that one can be certain that the drug is systemically present while behavioral studies are being conducted. The method described below was developed to determine microgram amounts of pemoline in serum and urine.

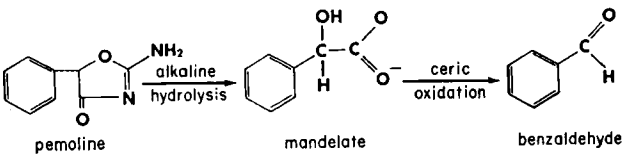
Although the method was designed specifically for the determination of pemoline, it can also be used to determine serum and urine levels of mandelic acid. This compound is of pharmaceutical interest since salts of mandelic acid are used to treat urinary tract infections.

To date, the only published methods for determination of mandelic acid in biological material are polarographic methods (2–4) and a gravimetric method (5). Due to their lack of sensitivity, these methods would not be applicable for blood level studies.

Although methods for assaying pemoline are available (6, 7), they are not sensitive enough to determine the microgram amounts present in biological material.

In this assay, pemoline is hydrolyzed under alkaline conditions to mandelic acid which is then oxidized to benzaldehyde with ceric sulfate and determined spectrophotometrically (Scheme I).

The final oxidation step of converting compounds to benzaldehyde has been employed for other drugs using periodate as the oxidant (8–11). In this study it was necessary to choose an oxidant with a greater oxidation potential, since less potent oxidants gave poor results when the oxidations were carried out in the presence of interfering biological compounds.



Scheme I—Conversion of Pemoline to Benzaldehyde

The procedure for urine analysis is somewhat more time-consuming than for serum assays, since an additional clean-up step utilizing a cation-exchange resin was required. However, in either case, the method is simple, rapid, and has a lower limit of sensitivity of about 3.0 mcg. for serum analysis and about 10 mcg. for the urine analysis.

For the determination of mandelic acid the same procedure described for the pemoline determination was followed, including the hydrolysis step.

EXPERIMENTAL

Apparatus—A spectrophotometer[3] equipped with microcells was used for these studies. Ion-exchange columns used in the urine assay were specially constructed so that the upper portion of the column was 12.5 cm. long with an inside diameter of 1.5 cm. and the lower tip was 5.0 cm. long with an inside diameter of 3 mm. Sintered-glass filters were fused into the lower tip of the 3-mm. diameter tube.

The only glassware needed in addition to the columns are 20- and 50-ml. glass-stoppered tubes.

Reagents—The following solutions were prepared from analytical grade reagents; trichloracetic acid 50%; NaOH 10 *N;* NaOH 8 *N;* HCl 8 *N;* HCl 1 *N;* *n*-hexane spectroquality; cation-exchange resin[4] (200–400 mesh); and ceric sulfate 0.20 *M* prepared by dissolving 126.5 g. of $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ in 1.0 l. of 7 *M* H_2SO_4 with stirring for about 30 min. The reagent was allowed to stand overnight and the insoluble precipitate, probably ceric phosphate, was removed by filtration.

Resin Preparations—The resin was washed twice with distilled water to remove fines and then placed in the column to give a resin bed 3.5 cm. high with a diameter of 3 mm. Five milliliters of 1 *N* HCl was then passed through the resin followed by 5.0 ml. of distilled water before the sample was placed on the column.

Method for Serum Assay—Proteins were precipitated from 7.0 ml. of either serum or plasma with 1.0 ml. of 50% TCA. Following centrifugation 5.0 ml. of the supernatant was removed, placed in a 20-ml. glass-stoppered test tube, and made alkaline with 1.0 ml. of 10 *N* NaOH to give a pH greater than 13. The contents were then hydrolyzed by heating the stoppered tubes for 20 min. in a boiling-water bath. After cooling the pH was lowered to approximately 1 with 1.0 ml. of 8 *N* HCl, followed by the addition of 2.0 ml. of the ceric sulfate reagent. The contents were mixed

[1] Abbott 13397 2-amino-5-phenyl-2-oxazolin-4-one.
[2] Cylert (Abbott 30400), an equimolar combination of pemoline and magnesium hydroxide, Abbott Laboratories, North Chicago, Ill.
[3] Zeiss PMQ II.
[4] Dowex 50-X8.

and allowed to stand in subdued light for 20 min. The solution was then made alkaline with 2.0 ml. of 10 *N* NaOH, and the benzaldehyde was extracted by shaking with 1.5 ml. of hexane for 10 min. Following centrifugation approximately 1.0 ml. of the hexane layer was removed and the absorbance of the extract was determined at 241 mμ using a control serum sample as a blank. Standards carried through the entire procedure were control serum samples containing known amounts of pemoline. The standard curves thus obtained were linear over a concentration range of 0–50 mcg. The method also appears to be very reproducible in that the mean absorbance for 18 different analyses, run on different days, of a 20-mcg. serum standard was 0.381 ± 0.009.

Employing this method the mean absorbance for 40 control human serum samples (7 ml.) was 0.060 ± 0.010. Peak serum levels following a 75-mg. oral capsular dose of pemoline occurred at 2 hr. and the mean for 15 subjects was 1.86 ± 0.27 mcg./ml. At this dose, serum levels can easily be measured up to 32 hr. following drug administration.

Precautions—The above method is applicable for the analysis of both serum and plasma. However, it is recommended that serum be used in preference to plasma because benzyl alcohol, a preservative in commercial preparations of heparin, and citrate, a commonly used anticoagulant, both interfere with the assay.

Method for Urine Assay—Three milliliters of urine were made alkaline with 3.0 ml. of 8 *N* NaOH and hydrolyzed by heating in stoppered tubes for 1 hr. in a boiling-water bath. Following hydrolysis the samples were cooled and solid material was removed by centrifugation. The supernatant was placed on the washed resin column and pressure was applied to give a flow rate ranging from 0.5 to 1.0 ml./min. This eluate plus a 4-ml. water wash was collected in a 50-ml. glass-stoppered tube and acidified with 1.5 ml. of 8 *N* HCl. Two milliliters of the ceric sulfate solution was added, and the tubes were gently agitated in subdued light for 10 min. The pH of the solution was then adjusted to approximately 11 with 2.0 ml. of 8 *N* NaOH and extracted by shaking with 8.0 ml. *n*-hexane for 10 min. Following centrifugation the absorbance of the benzaldehyde in the hexane layer was determined at 241 mμ using a similar extract of a predrug urine sample as a blank. Predrug urine samples containing known amounts of pemoline were carried through the entire procedure. These standards were linear over a concentration range of 0 to 100 mcg. The urine analyses, like the serum analyses, also appear to be very reproducible since a mean absorbance for 27 different analyses (run on different days) of a 50-mcg. urine standard was 0.432 ± 0.018.

With the above method the mean absorbance for 32 human control urine samples was 0.061 ± 0.028. Urine levels of pemoline for 16 subjects were determined following a 75-mg. oral capsular dose, and the mean total excretion level for 0–56-hr. interval was 49.14 ± 13.36 mg.

DISCUSSION

The described method has been employed in this laboratory for more than a year to acquire information on serum and urine levels of pemoline. It has also been demonstrated that the method is capable of determining microgram amounts of mandelic acid in both urine and serum. With both of these compounds very satisfactory data were obtained. All data presented in this paper to demonstrate the accuracy and reproducibility of the assay were obtained with pemoline and not with mandelic acid. Since pemoline is first converted to mandelic acid, it would be repetitious to present similar data on mandelic acid. The method has also been used to determine serum levels of benzyl alcohol. However, the results were not as satisfactory as those with pemoline and mandelic acid and, until additional work is done, the method should not be used to determine benzyl alcohol in biological fluids.

Evidently there are one or more substances (pro-oxidants) in urine, but not in serum, that catalyze the oxidation to benzoic acid. This is the reason for the use of the ion-exchange resin in the urine procedure. This purification procedure does two things: (*a*) it removes or at least partially removes the pro-oxidants and (*b*) it also removes UV-absorbing materials, thus yielding a lower background absorbance. The only thing known about these pro-oxidants is that these are cationic-type compounds since treatment of the urine sample with an anion-exchange resin would not remove these compounds. Possibly trace amounts of metals could be causing this problem. That benzaldehyde readily undergoes oxidation to benzoic acid in air, particularly in the presence of trace amounts of cations, is well known.

Although the alkaline hydrolysis step was initially designed to convert pemoline to mandelic acid, it was later discovered that it also lowers the background absorbance, presumably by converting nonpolar compounds to polar compounds which are not extracted with the hexane. The time of hydrolysis of 20 min. appears to be sufficient for the assay in serum but 1 hr. is necessary for the urine assay. Under these conditions, it is possible to obtain a reproducible as well as a low background spectrum.

Evidence that the hydrolysis converted pemoline to mandelic acid was obtained with TLC. Evidence that mandelic acid was converted to benzaldehyde was obtained by analyzing the hexane extracts on a recording spectrophotometer.[5]

No attempt was made to determine the exact amount of pemoline recovered from urine and serum. However, upon comparing standard curves from aqueous standards with those of the standard urine and serum samples, it appears that greater than 80% of the pemoline was recovered from the biological fluids.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 1, 1968, from the *Department of Pharmacology, Abbott Laboratories, North Chicago, IL 60064*

Accepted for publication December 23, 1968.

[5] Cary.