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**Keyphrases**

Drug stability in solid dosage forms
Vitamin A decay
Vitamin E decay
Equilibrium phenomena
UV analysis
TLC determination of free tocopherol

# *p*-Methoxycinnamate and Its Metabolite in Rabbit Serum

By WON SICK WOO

**A method of *p*-methoxycinnamate determination in serum is described. *p*-Methoxycinnamate, following intravenous injection, disappeared very rapidly from the serum with a half-life of 0.4 hr. due to metabolic alteration. When orally administered to fasted rabbits, it is rapidly absorbed and maximum concentration is reached within 1 hr. Maximum concentration proportionally increases with increasing dose. The metabolite of *p*-methoxycinnamate in serum was identified as *p*-methoxybenzoate.**

SCHROPHULARIAE RADIX, a medicinal plant cultivated in Korea, has long been used as an antipyretic and anti-inflammatory drug. In this laboratory, *p*-methoxycinnamic acid (*p*-MCA) was isolated from this plant, and its antipyretic and analgesic properties have been reported (1, 2). It was also shown that *p*-MCA decreased the ascorbic acid contents of adrenals of rats (3) and in unpublished experiments it has been shown that it inhibits the edema formation by irritants such as yeast, formalin, croton oil, and dextran. When administered to a human and rabbits, it is oxidized to *p*-methoxybenzoic acid (*p*-MBA) which is excreted in the urine as conjugates of glycine and glucuronic acid (4).

The present paper describes the rate of decline of the serum *p*-MCA concentration in rabbits following a single dose, and its metabolite in serum. A rapid and convenient method for the measurement of *p*-MCA in serum is included.

## EXPERIMENTAL

**Estimation of p-Methoxycinnamate in Serum**—The determination method of *p*-MCA has not yet been reported. The following UV spectrophotometry for measurement of *p*-MCA in serum was established.

Serum sample (0.2 ml.) was pipeted into a 5-ml. volumetric flask, brought to exactly 5 ml. by adding a 5% solution of $HClO_4$, and thoroughly mixed. After heating at 60–70° for 10 min., the mixture was cooled and centrifuged at 3000 r.p.m. for 5 min. The clear supernatant was poured into a 1-cm. silica cell and the absorbance was measured at a wavelength of 308 mμ in a Beckman DU spectrophotometer. The test solution was read against a 5% solution of $HClO_4$. The mean absorption of serum was subtracted from the absorbance readings. The mean absorption was determined on a series of sera from normal untreated animals.

Figure 1 shows the standard curve obtained by this method as applied to standard solution of sodium *p*-MCA. Linear function is clearly demonstrated over the range studied.

Table I illustrates the recovery of added amounts of sodium *p*-MCA to rabbit serum. The amounts recovered vary from 96 to 112%, averaging 100.5%.

When serum contained more than 20 mg. % sodium *p*-MCA, the analysis was carried out using a smaller aliquot of sample plus water, and diluting

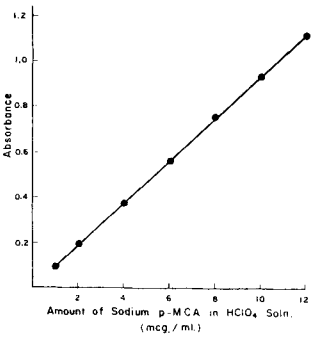


*Fig. 1—Calibration curve for Na p-MCA.*

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TABLE I—RECOVERY TEST OF SODIUM *p*-MCA IN SERUM

| Serum No. | Added, mg./100 ml. | Found, mg./100 ml. | Recovered, % |
|---|---|---|---|
| 1 | 12.750 | 12.500 | 98.04 |
| 2 | 12.750 | 13.000 | 101.96 |
| 3 | 12.750 | 13.125 | 102.94 |
| 4 | 12.750 | 12.250 | 96.08 |
| 5 | 12.750 | 12.750 | 100.00 |
| 6 | 6.375 | 6.250 | 98.04 |
| 7 | 6.375 | 7.125 | 111.76 |
| 8 | 6.375 | 6.425 | 100.78 |
| 9 | 6.375 | 6.325 | 99.22 |
| 10 | 6.375 | 6.150 | 96.47 |

supernatant with 5% $HClO_4$ if necessary, in order to make final concentration in $HClO_4$ solution less than 8 mcg./ml. The results were calculated from the following formula:

$$G \times V/v \times 0.1 = \text{mg. Na } p\text{-MCA/100 ml. serum}$$

where $G$ = amount (mcg.) found in Fig. 1 (after subtraction of serum absorption); $V$ = total volume of the solution after adding $HClO_4$; $v$ = volume of serum used.

**Experimental Animals**—All rabbits, weighing 2.0–2.5 Kg., were maintained on a constant diet for 1 week prior to the experiments and randomly divided into groups of 4–6 rabbits each. The indicated amounts of sodium *p*-MCA in water solution were administered intravenously and orally to rabbits. In the case of oral administration, animals had been fasted overnight. No food was ingested by animals until at least 5 hr. after drug administration.

**Extraction of Metabolites from Serum and Paper Chromatography**—A portion of the serum, at a definite interval after drug administration, was adjusted to pH 1 by adding dilute HCl, and extracted with ether. The ether extract was shaken with saturated aqueous solution of $NaHCO_3$. The aqueous phase was made to pH 1 again, and extracted with ether. After drying over anhydrous $Na_2SO_4$, ether was evaporated under reduced pressure from the extracts. The UV absorption spectrum of the residue in a 5% solution of $HClO_4$ was quite similar to that of the unextracted deproteinized serum.

The residue was dissolved in ethanol to use as a sample for paper chromatography. $R_f$ values of reference compounds are given in Table II.

TABLE II—CHROMATOGRAPHIC BEHAVIOR OF *p*-MCA AND RELATED SUBSTANCES[a]

| Compd. | $R_f$ | Color with Reagent A | B | C |
|---|---|---|---|---|
| *p*-Methoxycinnamic acid | 0.65 | Bl | Br | ... |
| *p*-Methoxybenzoic acid | 0.58 | Bl | ... | ... |
| *p*-Methoxycinnamoyl glycine | 0.57 | Bl | Br | Or |
| *p*-Methoxybenzoyl glycine | 0.53 | Bl | ... | Or |
| *p*-Hydroxycinnamic acid | 0.35 | Bl | Br | ... |
| *p*-Hydroxybenzoic acid | 0.25 | Bl | Br | ... |

[a] The ascending method was used with Whatman No. 4 paper at room temperature. Solvent mixture: isopropyl alcohol–$NH_4OH$–$H_2O$–toluene (80:5:15:20). Detecting reagents: *A*, 0.4% bromophenol blue; *B*, iodine vapor; *C*, 4% *p*-dimethylaminobenzaldehyde in acetic anhydride. Abbreviations: Bl, blue; Br, brown; Or, orange.

*p*-Methoxycinnamoyl glycine (*p*-MCG), m.p. 161°, and *p*-methoxybenzoyl glycine (*p*-MBG), m.p. 172°, were prepared by reaction of corresponding acid chlorides with glycine. *p*-MCA and *p*-hydroxycinnamic acid (*p*-HCA) were prepared according to Knoevenagel (5) and Zincke and Leisse (6), respectively, and *p*-MBA by oxidation of anisaldehyde. *p*-Hydroxybenzoic acid (*p*-HBA) was obtained commercially.

## RESULTS AND DISCUSSION

**The Decline of Serum p-Methoxycinnamate Concentration After Intravenous Administration**—Figure 2 shows the decline of the serum *p*-MCA concentration following intravenous administration at dose levels of 100 mg. and 200 mg./Kg. Serum *p*-MCA concentrations were about 41 mg. and 80 mg./100 ml. 3 min. after injection. It can be seen that *p*-MCA disappeared very rapidly from serum and this elimination was first order. Half-life time for excretion was about 0.4 hr. But this first-order process was preceded by an initial apparent lag phase.

**Variation of the Serum p-Methoxycinnamate Concentration After Oral Administration**—The results obtained in this study are given as plots of log concentration against time in Fig. 3. The peaks of serum concentration were obtained at around 30–60 min. after drug administration. This result indicates that *p*-MCA was rapidly absorbed. Since *p*-MCA could not be detected in feces excreted within 24 hr. after drug administration (7), it is suggested that *p*-MCA was almost completely absorbed from the digestive tract. The elimination of *p*-MCA from serum was found to proceed by first-order kinetics at a small dose (100 mg./Kg.). At higher doses, *p*-MCA elimination did not follow first-order kinetics (nor a zero-order process), until the amount remaining in the serum was decreased to about 17 mg./100 ml. When serum *p*-MCA concentration has fallen below this level, the first-order elimination seemed to occur. But the rate constants were essentially the same, regardless of

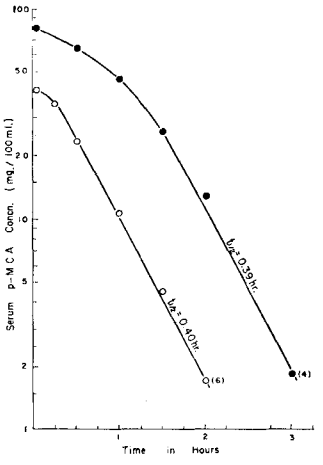


*Fig. 2—Elimination of* p-*MCA from serum following its intravenous injection of 100 mg./Kg.* (○) *and 200 mg./Kg.* (●). *Figure in parentheses at the right of each line is number of rabbits used.*

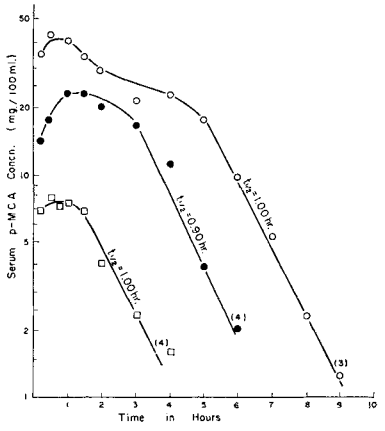


*Fig. 3—Serum* p-*MCA levels after its oral administration. Key:* □, *100 mg./Kg.;* ●, *300 mg./Kg.;* ○, *500 mg./Kg. Figure in parentheses at the right of each line is number of rabbits used.*

the amount of drug administered. This observed phenomenon is similar to that of salicylate elimination at higher doses (8).

It is of interest that half-life for first-order elimination when administered orally differed from that when administered intravenously. In the case of administration of 100 mg./Kg., the *p*-MCA level was higher (10.85 mg. ± 2.0/100 ml.) 1 hr. after intravenous treatment than when orally given (7.38 mg. ± 0.41/100 ml.). After 2 hr., the *p*-MCA level was higher (3.97 mg. ± 0.8/100 ml.) by oral administration than by intravenous (1.68 mg. ± 0.51/100 ml.).

**Maximum Serum p-Methoxycinnamate Concentration as Function of Dose**—By representing the average value of observed serum concentration at 30–60 min. after oral administration of drug as a mean maximum serum concentration, over-all results are given in Fig. 4, which indicate that there is a linear relationship between mean maximum concentration and dose.

**Metabolite of p-Methoxycinnamate in Serum**—Figure 5 shows the spectra of deproteinized serum with $HClO_4$ at various times after an intravenous injection of 100 mg./Kg. Absorption peak at 308 mμ might represent that of *p*-MCA. Although *p*-MCG and *p*-HCA have the absorption peaks at around 292 mμ and 306 mμ, respectively, as shown in Fig. 6, they were not detected in serum by paper chromatography. It is, therefore, evident that this peak was reflected neither by *p*-MCG nor by *p*-HCA. *p*-MBA, *p*-MBG, and *p*-HBA all have no absorbance above 300 mμ. These facts reconfirm that UV spectrophotometry of *p*-MCA is suitable for its determination in serum.

This absorption peak at 308 mμ decreased considerably as time passed. However, the secondary peak at around 258 mμ developed, which also decreased with advance of time. From this phenomenon it is clear that *p*-MCA was converted into another substance. The fact that *p*-MBA has an absorption peak at 258 mμ as shown in Fig. 6 and that *p*-MBA was detected in serum by paper chromatography while neither *p*-MBG nor *p*-HBA was detected indicates that the secondary peak might be identical with that of *p*-MBA. These results show that *p*-MCA is *in vivo* rapidly metabolized to *p*-MBA. This fact coincides with the previously reported communication (4).

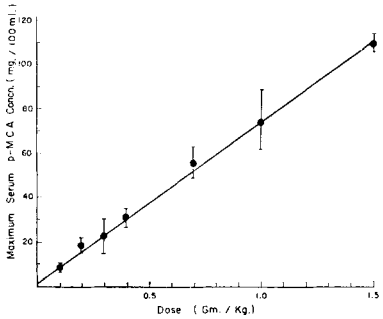


*Fig. 4—Linear relationship between mean maximum serum concentration of* p-*MCA and oral dose.*

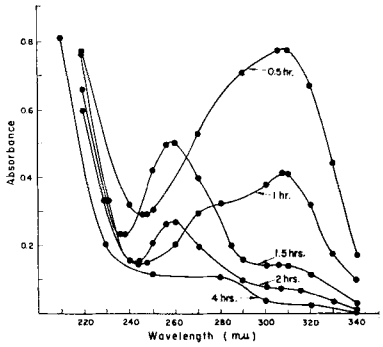


*Fig. 5—Typical UV absorption spectra of deproteinized serum at various times after an intravenous injection of 100 mg./Kg. of* p-*MCA.*

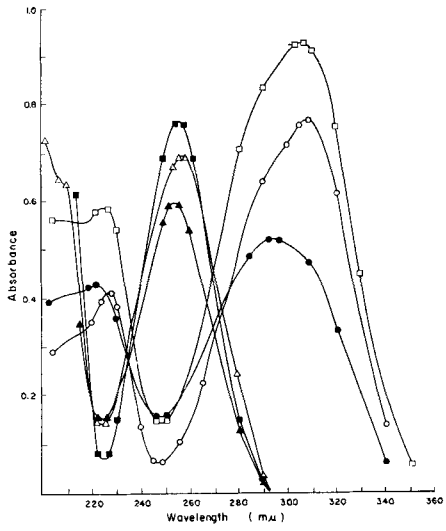


*Fig. 6—UV absorption spectra of* p-*MCA and its related compounds in 5% $HClO_4$. Key:* ○, p-*MCA (8.3 mcg./ml.);* ●, p-*MCG (8.8 mcg./ml.);* □, p-*HCA (9.6 mcg./ml.);* ■, p-*HBA (8.0 mcg./ml.);* △, p-*MBA (8.0 mcg./ml.);* ▲, p-*MBG (8.0 mcg./ml.).*

## SUMMARY

A rapid and convenient method was developed for the measurement of *p*-methoxycinnamate. With this method, the serum *p*-methoxycinnamate concentration has been measured after oral and intravenous administration to rabbits. *p*-Methoxycinnamate disappeared very rapidly from serum with a half-life of 0.4 hr. when injected intravenously. When orally administered, it was rapidly absorbed into the blood stream, and blood levels peaked within 1 hr. but then declined slowly. Average maximum concentrations were strictly proportional to the oral doses. Its elimination from serum was also first order, but the apparent half-life was more than 2 times that when administered intravenously. At higher doses, however, the elimination was not an exponential process in the initial stage. The metabolite of *p*-methoxycinnamate was identified as *p*-methoxybenzoate by ultraviolet spectrophotometry and paper chromatography.



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*Keyphrases*

*p*-Methoxycinnamate
Serum levels of *p*-methoxycinnamate
*p*-Methoxybenzoate identified as metabolite
UV analysis
Paper chromatographic analysis
Half-life of *p*-methoxycinnamate


# Opium Alkaloids VI

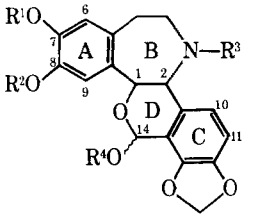
## Isolation of *N*-Methyl-14-*O*-desmethylepiporphyroxine


By E. BROCHMANN-HANSSEN, K. HIRAI, B. NIELSEN, S. PFEIFER, I. MANN, and L. KÜHN



**The papaverrubine alkaloids and their *N*-methyl derivatives are widely distributed in the genus *Papaver*. They have a number of interesting structural and stereochemical characteristics. A new alkaloid of this general class has now been isolated from opium. It has been characterized by means of NMR and mass spectrometry and by chemical conversions to known compounds.**


ALKALOIDS of the general structure (I) are widely distributed in the genus *Papaver* and actually constitute the principal alkaloids of several species (1–3). Many compounds of this type have been isolated from natural sources and differ with regard to their substitution pattern, their conformation, as well as the stereochemistry at the three asymmetric centers. Four opium alkaloids belonging to this group have been reported, namely porphyroxine = papaverrubine D (I; $R^1 = R^4 = CH_3$, $R^2 = R^3 = H$) (4–8), papaverrubine B = *O*-methylporphyroxine (I; $R^1 = R^2 = R^4 = CH_3$, $R^3 = H$) (6, 7, 9, 10), papaverrubine C = epiporphyroxine (I; $R^1 = R^4 = CH_3$, $R^2 = R^3 = H$) (12), and glaudine = *O,N*-dimethylporphyroxine (I; $R^1 = R^2 = R^3 = R^4 = CH_3$) (9, 11). They have a *trans*-

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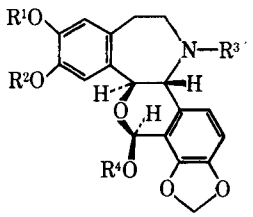


II

configuration at the B/D ring junction, whereas other bases of this type, *e.g.*, rhoeadine from


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