Michaelis–Menten Kinetics of Renal Tubular Secretion of D-(−)-*p*-Methyl Mandelic Acid and D-(−)-*p*-Ethyl Mandelic Acid in Rats

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Abstract □ Studies were carried out to determine the Michaelis–Menten kinetic parameters (V_m and K_m) of renal tubular secretion of *para*-alkylated mandelic acids, namely, D-(−)-*p*-methyl mandelic acid and D-(−)-*p*-ethyl mandelic acid, in rats. It is shown that the maximum initial secretion rates (V_m) of these compounds are similar, and they share a common "carrier" mechanism for their renal tubular secretion. Since the value of the intravenous dose (μmole/kg.) required to produce half the maximum secretion rate (K_m) for D-(−)-*p*-ethyl mandelic acid is found to be about one-half that for D-(−)-*p*-methyl mandelic acid, it is concluded that the affinity of the former for the "carrier" is approximately twice that of the latter. Evidence was obtained to indicate that the *para*-alkylated mandelic acids used in this study are secreted by the same mechanism responsible for the secretion of D-(−)-mandelic acid and its homologs such as tropic acid. Although the volume of distribution available in rats for these *para*-alkylated mandelic acids is demonstrated to be similar, it is shown to be strikingly smaller than that for D-(−)-mandelic acid and its homologs obtained by introducing methylene group(s) in the side chain of the mandelic acid molecule. The biological significance of the data obtained for the "model compounds" is discussed.

Keyphrases □ D-(−)-*p*-Methyl- and D-(−)-*p*-ethyl mandelic acids—renal tubular secretion □ Renal tubular secretion—*p*-alkylated mandelic acids □ Kinetic parameters, Michaelis-Menten—renal tubular secretion, *p*-alkylated mandelic acids □ Absolute configuration—methyl and ethyl mandelic acids □ GLC—analysis

Recent studies in rats from this laboratory (1) showed that the introduction of methylene group(s) in the side chain of a model compound, D-(−)-mandelic acid, decreases the biological half-lives of the compounds as compared to that of the parent compound. The various compounds studied were D-(−)-4-hydroxy-4-phenylbutanoic acid, D-(−)-benzyllactic acid, L-(+)- and D-(−)-tropic acid, and D-(+)- and L-(−)-phenyllactic acid. Furthermore, these compounds were shown to exhibit a mutual inhibitory effect on their renal tubular secretion and to share a common "carrier" mechanism for their secretion (1). Through the Michaelis–Menten kinetic treatment of the initial rates of renal tubular secretion data obtained for D-(−)-mandelic acid (hereafter referred to as mandelic acid) and DL-tropic acid (hereafter referred to as tropic acid), it was demonstrated that these compounds have similar maximum initial secretion rates and that the affinity of tropic acid for the "carrier" is approximately twice that of mandelic acid (2). In addition to being completely excreted unchanged in the urine of rats, since mandelic acid and its above mentioned homologs are neither metabolized nor bound to the plasma proteins, these compounds of low pKa values (3.7–4.7) were considered as the model compounds in the context of the objective of the studies (1, 2). The kinetic studies of urinary excretion of these model compounds were carried out to distinguish certain structural characteristics around the positively charged site of the carrier molecules believed to be present in the renal tubular membrane of rats for secretion of the anionic drugs.

The purposes of the work presented here were: (*a*) to study the kinetics of urinary excretion of the *para*-alkylated mandelic acids, namely, D-(−)-*p*-methyl mandelic acid and D-(−)-*p*-ethyl mandelic acid (hereafter referred to as methyl mandelic acid and ethyl mandelic acid), in rats, and (*b*) to determine if these compounds also share the same "carrier" for their renal tubular secretion as do mandelic acid and its above mentioned homologs. These objectives were accomplished by studying the Michaelis–Menten kinetics of renal tubular secretion of these compounds in rats.

EXPERIMENTAL

Materials—The following were used: D-(−)-mandelic acid[1], m.p. 132–133°, $[\alpha]_D^{25}$ − 149° [2]; D-(−)-*p*-methyl mandelic acid, m.p. 130–131°, $[\alpha]_D^{25}$ − 159°[2], obtained by resolving from the racemic acid synthesized by the method of Corsow *et al.* (3); D-(−)-ethyl mandelic acid, m.p. 119–120°, $[\alpha]_D^{25}$ − 120°[2], obtained by resolving from the racemic acid synthesized by the method of Klingenberg (4); and DL-tropic acid, m.p. 118–119°.

Apparatus—Mandelic acid and its homologs appearing in the urine of rats were quantitated by the gas chromatographic method previously described (1, 2), using 5% ethylene glycol succinate as well as 15% silicone rubber as the liquid phases. The specific rotation of the compounds was determined with the aid of a Perkin-Elmer 141 polarimeter, using a 1-ml. capacity polarimeter tube. The urine pH determinations were made on a Beckman model 76 Expandomatic pH-meter. The optical rotatory dispersion (ORD) curves of the compounds were obtained with the aid of a Cary model 60 recording spectropolarimeter.

Methodology—The procedure employed for preparing the rats for the study and for urine collection following the intravenous administration of the compounds was the same as previously described (1). About 30 Sprague-Dawley male rats, weighing between 185 and 235 g., were repeatedly used. None of the rats was used more than five times in the studies, and the rest period allowed between the successive use of a rat was about 2 weeks. The aqueous solutions of sodium salts of each compound used for intravenous administration were made isotonic, and the volume of intravenous injections was 2 ml. The urinary excretion kinetics of methyl mandelic acid were studied over the dosage range of 1.43–15.06 × 10^2 μmoles/kg.[3] (5–50 mg. per rat) to determine the dependency of the biological half-life of the compound on its intravenous dose. The urinary excretion kinetics of ethyl mandelic acid were studied over the intravenous dosage range of 1.27–3.94 × 10^2 μmoles/kg.[4] (5–15 mg. per rat).

To study the Michaelis–Menten kinetics of renal tubular secretion of the compounds in rats, their initial glomerular filtration rates (and, consequently, the initial secretion rates) were determined at the appropriate dosage levels in the presence of suitable competitive

[1] Aldrich Chemical Co., Milwaukee, Wis.

[2] For specific rotation determinations, the solutions of these compounds were prepared in 95% ethanol.

[3] Doses used were 143, 172, 320, 342, 391, 430, 512, 512, 523, 573, 573, 885, 903, 1420, and 1506 μmoles/kg.

[4] Doses used were 126, 149, 218, 222, 230, 288, 300, 377, and 391 μmoles/kg.

Table I—Conditions Employed for Gas Chromatographic Analysis and Retention Times Observed for the Compounds

	Oven Temperature[a]	He Flow Rate, ml./min.	Retention Time, min.
Methyl-*p*-methyl mandelate	180°	40	5.8
Methyl-*p*-ethyl mandelate	180°	40	7.2
Methyl-*p*-ethyl mandelate	160°	40	13.5[b]

[a] The injection port and detector temperatures were maintained at 210°. [b] As stated in the text, 15% silicone rubber was used as a liquid phase column to analyze methyl-*p*-ethyl mandelate in the presence of methyl tropate.

inhibitors of secretion, according to the method previously described (2). To determine the initial glomerular filtration rates of methyl mandelic acid at the dosage levels of 1.36–4.52 × 10^2 μmoles/kg. (5–15 mg. per rat) i.v., the intravenous dose of the sodium salt of the compound was injected into the rat 20 min. after the administration of a 5.46–13.36 × 10^3-μmoles/kg. (200–400 mg. per rat) i.p. dose of tropic acid as the sodium salt contained in 5 ml. of water at a pH of about 7.0. In a similar manner, the apparent initial glomerular filtration rates of ethyl mandelic acid at the dosage levels of 2.45–3.03 × 10^2 μmoles/kg. (8–10 mg. per rat) i.v. were determined in the presence of a 12.05 × 10^3-μmoles/kg. (400 mg. per rat) i.p. dose of methyl mandelate or 11.92–11.98 × 10^3-μmoles/kg. (400 mg. per rat) i.p. dose of tropic acid. The initial glomerular filtration rates of ethyl mandelic acid were also determined in the presence of simultaneously administered 25.74–26.02 × 10^2-μmoles/kg. (80 mg. per rat) i.v. doses of methyl mandelic acid.

The intravenous doses of sodium methyl mandelate and sodium ethyl mandelate employed in the studies designed to determine the maximum initial secretion rates (V_m), as well as their doses required to produce one-half of the maximum initial secretion rates

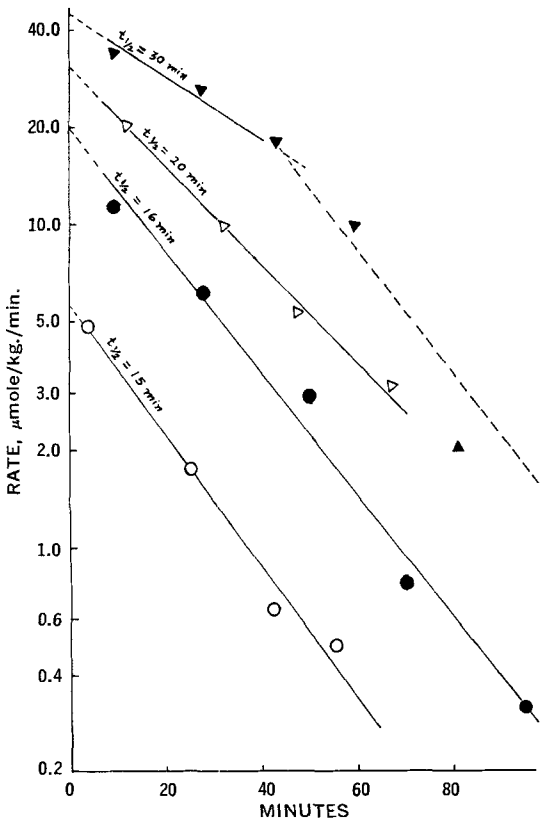

Figure 1—*Apparent first-order urinary excretion of* D-(−)-p-*methyl mandelic acid, showing the dependence of biological half-life* ($t_{1/2}$) *of the compound on the intravenous dose. Key:* ○, *143 μmoles/kg.;* ●, *512 μmoles/kg.;* △, *885 μmoles/kg.; and* ▲, *1506 μmoles/kg.*

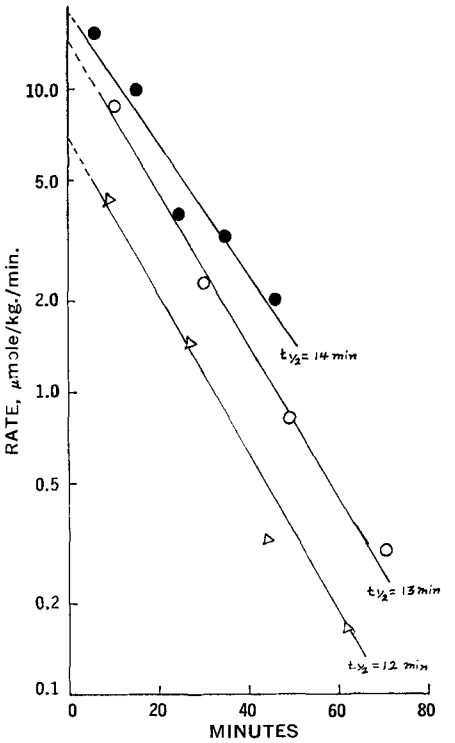

Figure 2—*Apparent first-order kinetics of urinary excretion of* D-(−)-p-*ethyl mandelic acid following its intravenous administration of 126-μmoles/kg.* (△), *288-μmoles/kg.* (○), *and 377-μmoles/kg.* (●) *doses to rats.*

(K_m), were in the range of 1.43–15.06 × 10^2 and 1.27–3.94 × 10^2 μmoles/kg., respectively.

To determine whether the inhibition of renal tubular secretion of methyl mandelic acid by tropic acid is competitive or noncompetitive, the initial secretion rates of methyl mandelic acid (dosage range 2.94–9.03 × 10^2 μmoles/kg.) were determined in the presence of 21.39–24.09 × 10^2-μmoles/kg. (80 mg. per rat) i.v. doses of tropic acid. In these studies both the substrate and inhibitor compounds, contained in the same intravenous solution, were administered as their sodium salts.

Gas Chromatographic Analysis of the Compounds—The urine samples were quantitatively analyzed for mandelic acid, methyl mandelic acid, and ethyl mandelic acid by a gas chromatographic method previously described (1). The liquid phase column used for the gas chromatographic analysis of these compounds was 5% ethylene glycol succinate. However, a 15% silicone rubber liquid phase column was used to obtain a distinct peak for ethyl mandelic acid in the presence of methyl mandelic acid. The conditions employed for the gas chromatographic analysis and retention times observed for methyl esters of the compounds are described in Table I.

Determination of Dissociation Constants and Apparent Partition Coefficients of the Compounds and Their Binding to Whole Rat Blood—These determinations were carried out by the methods previously described for mandelic acid and its several homologs (1).

Determination of Absolute Configuration of the Compounds—The ORD curves for methyl mandelic acid and ethyl mandelic acid were obtained and compared with that obtained for D-(−)-mandelic acid.

RESULTS AND DISCUSSION

Selection of the Compounds—Various properties considered ideal for use of the compounds in view of the objective of the studies were previously discussed (1). Since it was observed in preliminary studies that, of the administered dose of 10–20 mg. i.v. per rat, approximately 58% of the racemic methyl mandelic acid and 40% of the racemic ethyl mandelic acid are recovered unchanged in the urine of rats, the racemic compounds were resolved into their levorotatory optical isomers and their urinary recovery was studied in rats. The preliminary studies showed that 90–100% of the ad-

ministered doses (10–20 mg. i.v. per rat) of the levorotatory isomers of each of the two compounds was excreted unchanged in the urine of rats. Therefore, the other properties (dissociation constants, partition coefficients, and binding to plasma proteins) of the compounds were studied to determine their suitability for use in the present study.

The pKa value determined for levorotatory methyl mandelic acid is 3.4, and that determined for levorotatory ethyl mandelic acid is also 3.4 (lit. 3.5). The partition coefficient ($C_{organic}/C_{buffer}$) observed for methyl mandelic acid as well as ethyl mandelic acid between the pH 6.5 phosphate buffer solution (10 ml. solution containing 5 mg. of the acid) and chloroform or ether (10 ml.) was zero, as was found previously for mandelic acid, tropic acid, or phenyllactic acid (1). The results of the binding studies indicated that, at equilibrium, the concentration of methyl mandelic acid or ethyl mandelic acid in the solution (4 ml.) outside of the dialysis bag (containing 4 ml. of whole rat blood) was the same as in the solution (4 ml.) outside of the dialysis bag (containing 4 ml. of pH 7.4 phosphate buffer solution) of the control dialysis setup, indicating that neither of the acids is bound to the plasma proteins of rat. The amount of either acid used per dialysis setup was 1 mg. (1). As previously noted (1), the pH of rat urine was generally found to be in the range of 6.5–8, even after administration of a 400-mg. i.p. dose of tropic acid to the rats. Since these compounds possessed these ideal properties, the levorotatory isomers of *p*-methyl mandelic acid and *p*-ethyl mandelic acid were considered as the model compounds for use in the present study.

The absolute configurations of the levorotatory isomers of methyl mandelic acid and ethyl mandelic acid were determined by comparing the ORD curves of the compounds with that of D-(−)-mandelic acid (1). Since the plain ORD curves obtained for these compounds were similar to that of D-(−)-mandelic acid over the wavelengths of 600–200 nm., D absolute configuration was assigned to the levorotatory isomers of these compounds.

Apparent First-Order Urinary Excretion of the Compounds—As observed for mandelic acid and its several homologs (1), the urinary excretion of methyl mandelic acid and ethyl mandelic acid was found to occur by an apparent first-order process in rats. Since these compounds are entirely excreted in the urine in the unchanged form, the urinary excretion data were treated according to the following equation (1):

$$\log \Delta A_e/\Delta t = \log kA_0 - kt/2.303 \qquad \text{(Eq. 1)}$$

where $\Delta A_e/\Delta t$ is the rate of excretion of the compound at time t, A_0 is the amount of the compound in the body at zero time, and k is the apparent first-order rate constant for excretion of the compound. The rate constant, k, was obtained from the slope ($-k/2.303$) of the straight line, obtained upon plotting $\log \Delta A_e/\Delta t$ *versus* t. The straight line in each case was obtained by the method of least squares. The time t in these plots represented the midpoints of the urine-collection intervals. The biological half-lives of the compounds were calculated from $0.693/k$.

As shown in Fig. 1, it was observed that, while the biological half-life for methyl mandelic acid remained reasonably constant (16 ± 1.7 min.) over the dosage range of 1.43–9.03 × 10^2 μmoles/kg. i.v., it increased with the increase in the dose above this dosage level during the initial period of about 40 min. This may mainly be due to the increased saturation of the renal tubular secretory process with the increase in the dose of the compound. Although the two kinetic studies were performed at high doses (1420 and 1506 μmoles/kg.), unfortunately, it did not become possible to obtain from the rats the urine samples at shorter intervals (12 min. or so) to characterize the data curve after 50 min. and thereby be able to indicate that the half-life of the compound became about 16 min. Nevertheless, data in Fig. 1 tend to indicate that, after the initial period, the compound was excreted at a faster rate (shorter biological half-life), as can be expected. The apparent initial excretion rates of the compound at various dosage levels were determined from the values of the intercepts obtained by extrapolating the straight lines to zero time (1). The apparent first-order urinary excretion data obtained for ethyl mandelic acid over the dosage range of 1.27–3.94 × 10^2 μmoles/kg. are shown in Fig. 2. The biological half-life of the compound over this dosage range was found to be fairly constant (13.5 ± 1.6). As shown later, since it was demonstrated that methyl mandelic acid and tropic acid are able to inhibit the secretion of ethyl mandelic acid and that the data obtained at these dosage levels of ethyl mandelic acid were sufficient to determine the Michaelis–Menten kinetic parameters of its secretion, it was not necessary to carry out additional studies at higher doses of the compound to show the saturation effect on its renal tubular secretory process, as was done in the case of methyl mandelic acid (Fig. 1).

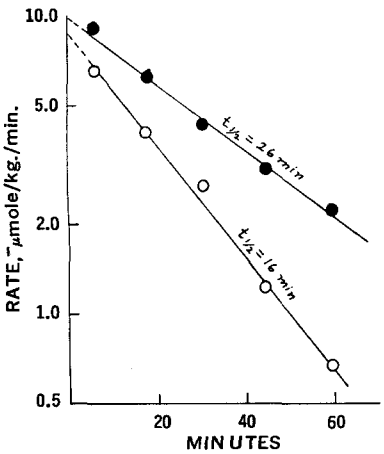

Figure 3—*Urinary excretion of* D-(—)-*mandelic acid* (●, *343 μmoles/kg.*) *and* D-(−)-*ethyl mandelic acid* (○, *219 μmoles/kg.*) *following their simultaneous intravenous administration to a rat.*

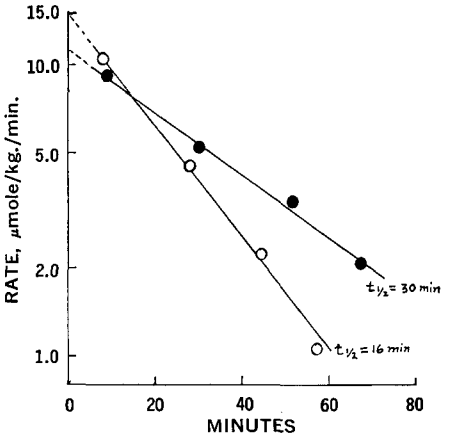

Figure 4—*Apparent first-order urinary excretion of* D-(−)-p-*methyl mandelic acid following intravenous administration of a 391-μmoles/kg. dose in the absence* (○) *and a 452-μmoles/kg. dose in the presence* (●) *of a 1.2* × 10^4-*μmoles/kg. i.p. dose of* DL-*tropic acid to rats.*

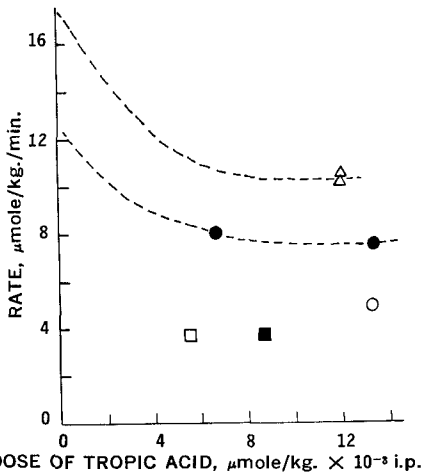

Figure 5—*Plots showing that the apparent limiting initial excretion rates* (i.e., *apparent initial glomerular filtration rates*) *of* D-(−)-p-*methyl mandelic acid* (*dosage range 136–452 μmoles/kg. i.v.*) *can be obtained in the presence of a 1.2* × 10^4-*μmoles/kg. i.p. dose of* DL-*tropic acid. Key:* □, *136 μmoles/kg.;* ■, *146 μmoles/kg.;* ○, *167 μmoles/kg.;* ●, *334 μmoles/kg.; and* △, *452 μmoles/kg.* (*The rates of the compound in the absence of tropic acid were obtained by extrapolating the data in Fig. 1.*)

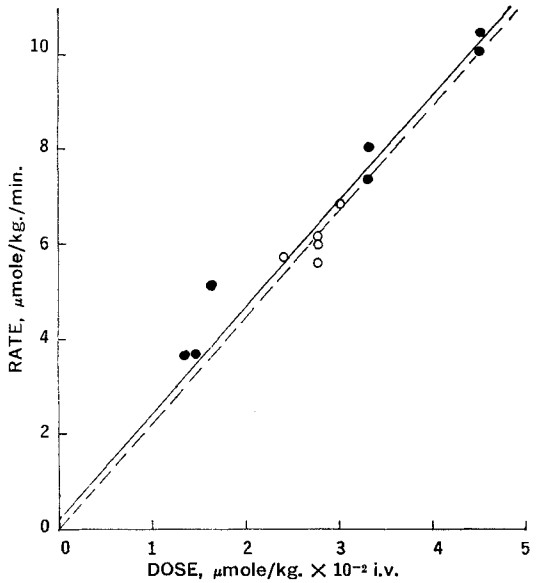

Figure 6—*Relationship between the apparent initial glomerular filtration rate and the intravenous dose for* D-(−)-p-*methyl mandelic acid* (●) *and* D-(−)-p-*ethyl mandelic acid* (○). (*The straight lines are obtained by the method of least squares.*)

The biological half-life of ethyl mandelic acid is found to be significantly shorter than that of methyl mandelic acid ($p < 0.01$).

The biological half-life observed for methyl mandelic acid or ethyl mandelic acid in this study is considerably shorter than that noted for mandelic acid (30 ± 4 min.) by Randinitis *et al.* (1) in most of their studies. However, it was noted that, while the volume of intravenous injection used in the present studies for the administration of various doses of the compounds to each rat was 2 ml., the volume of intravenous injection used by Randinitis *et al.* (1) for the administration of similar doses of mandelic acid to each rat varied from 0.2–0.7 ml. To make a valid comparison of the biological half-lives of these compounds, it was considered desirable to

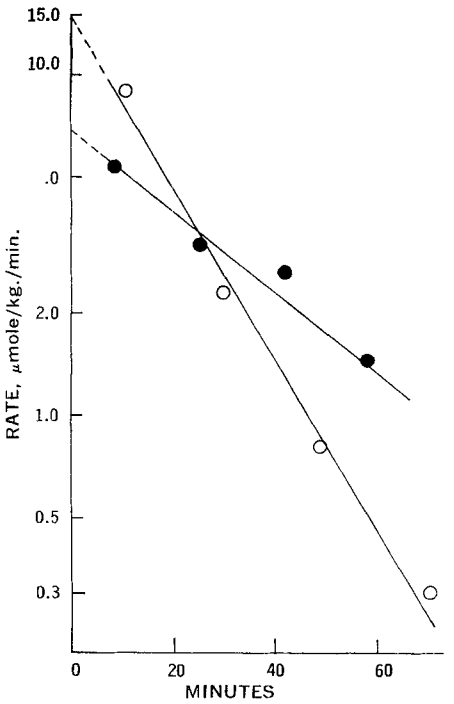

Figure 7—*Apparent first-order urinary excretion of* D-(−)-p-*ethyl mandelic acid following the intravenous administration of a 291-μmoles/kg. dose in the absence* (○) *and 303-μmoles/kg. dose in the presence* (●) *of a 2.6 × 10³-μmoles/kg. simultaneously administered intravenous dose of* D-(−)-p-*methyl mandelic acid to rats.*

Table II—Michaelis–Menten Kinetic Parameters of Renal Tubular Secretion for the Compounds in Rats

Compound	Apparent V_m, μmole/kg./min.	Apparent K_m, μmole/kg. × 10^{-2}
D-(−)-Methyl mandelic acid	21.6	10.2
D-(−)-*p*-Ethyl mandelic acid	18.5	4.9
D-(−)-Mandelic acid[a]	18.8	11.1
DL-Tropic acid[a]	20.8	5.2

[a] Values for these compounds are adapted from *Reference 2.*

determine an obvious influence of the injection volume on the biological half-lives of these compounds. In one study, the urinary excretion kinetics of mandelic acid (493 μmoles/kg.) were studied following its administration as the sodium salt contained in a 2-ml. i.v. injection volume; in the other study, the excretion kinetics of mandelic acid (343 μmoles/kg.) and ethyl mandelic acid (219 μmoles/kg.) were studied following their simultaneous administration as the sodium salts contained in a 2.5-ml. i.v. injection volume. The biological half-life determined for mandelic acid in the latter study was 26 min. (Fig. 3) and that determined in the former study was 32 min., both these values being within the range previously reported (1). The biological half-life of 16 min. observed for ethyl mandelic acid (Fig. 3) was equal to the uppermost limit value in the range noted in the present study. Therefore, no attempt was made to ascertain if mandelic acid had exerted a slight inhibitory effect on the renal tubular secretion of ethyl mandelic acid at these dosage levels. Since none of the homologs of mandelic acid previously studied (1) significantly influenced the biological half-life of mandelic acid at such low doses, it may be assumed that the half-life of mandelic acid was not influenced by ethyl mandelic acid in the study (Fig. 3). Thus, it seems that the injection volume (up to 2.5 ml.) has no apparent effect on the biological half-lives of the compounds.

As noted by Randinitis *et al.* (1) for mandelic acid and its homologs, in this study also the metabolism and the binding of methyl mandelic acid and ethyl mandelic acid to blood proteins of rats were negligible and their pKa values were considerably lower than the pH values of urine of rats. In view of these facts, the shorter biological half-lives observed for methyl mandelic acid and ethyl mandelic acid than that observed for mandelic acid may be attributed to the smaller volume of distribution available in rats for these compounds than that for mandelic acid and/or to the greater affinity of these compounds for the "carrier" for renal tubular secretion in rats than that of mandelic acid, if these compounds share a common "carrier" for their secretion. The influence of each factor is evaluated from the subsequent studies carried out to determine the initial glomerular filtration rates and the Michaelis–Menten kinetic parameters of renal tubular secretion of these compounds in rats.

Determination of Apparent Initial Glomerular Filtration Rates of the Compounds—In the studies designed to determine the initial glomerular filtration rates of methyl mandelic acid, the compound used to inhibit its secretion was tropic acid, which has a greater affinity for the renal tubular secretion "carrier" than that of mandelic acid (2). In the urinary excretion kinetic studies, the dose (5.46–13.36 × 10^3 μmoles/kg. i.p.) of tropic acid was administered 20 min. prior to the intravenous administration of methyl mandelic acid to allow substantial absorption of the inhibitor dose from the site of injection (1). As shown in Fig. 4, the biological half-life of methyl mandelic acid increased[5], and its initial excretion rate decreased, in the presence of tropic acid. Also, as depicted in Fig. 5, it is seen that the 10–13 × 10^3-μmoles/kg. i.p. dose of tropic acid produced a limiting initial excretion rate of methyl mandelic acid over its dosage range of 136–452 μmoles/kg. i.v. Since a linear relationship (Fig. 6) was observed between the intravenous dose of methyl mandelic acid and its limiting initial excretion rate at the corresponding dose, the limiting initial excretion rates noted for the compound were considered as its apparent initial glomerular filtration rates at the corresponding dosage levels (2).

[5] The range of biological half-life values observed was 22–38 min., depending on the dose of tropic acid used as the inhibitor.

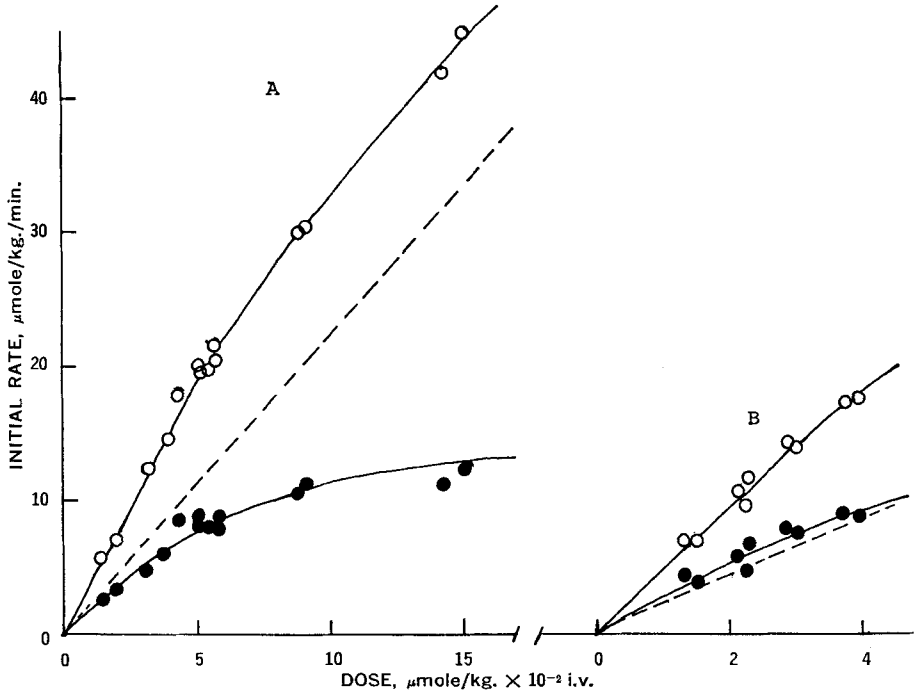

Figure 8—*Apparent initial excretion rates, ○; initial glomerular filtration rates, - - -; and initial secretion rates, ●; observed for* D-(−)-p-*methyl mandelic acid (A) and* D-(−)-p-*ethyl mandelic acid (B) following the intravenous administration of appropriate doses to rats.*

To determine the initial glomerular filtration rates of ethyl mandelic acid in rats, the compounds used as the inhibitors of its secretion were methyl mandelic acid and tropic acid. The purpose of using both these compounds as the inhibitors was to obtain additional evidence that all these compounds are secreted by a common "carrier" mechanism. Initially, two glomerular filtration-rate determination studies were carried out for the compound in the presence of 12.05×10^3-μmoles/kg. i.p. doses of methyl mandelic acid, which was administered to the rats 20 min. prior to the intravenous administration of the compound. But this dose of methyl mandelic acid caused bleeding in the urine of rats. Therefore, the additional studies were carried out using lower doses ($25.74–26.02 \times 10^2$ μmoles/kg. i.v.) of methyl mandelic acid as the inhibitor. In this event, both ethyl mandelic acid (dose of $2.45–3.03 \times 10^2$ μmoles/kg. i.v.) and methyl mandelic acid, contained in a 2-ml. intravenous injection volume as their sodium salts, were administered to the rats. When tropic acid was used as the inhibitor of secretion of methyl mandelic acid, a dose (12.0×10^3 μmoles/kg. i.p.) of the former was administered to the rats 20 min. prior to the administration of the intravenous dose of the latter. A typical kinetic study showing the inhibitory effect of methyl mandelic acid on the secretion of ethyl mandelic acid is shown in Fig. 7. When the apparent initial excretion rates obtained in these studies for ethyl mandelic acid were plotted against its corresponding intravenous doses, a linear relationship was observed (Fig. 6). This demonstrated that not only approximately 12×10^3-μmoles/kg. i.p. doses of methyl mandelic acid or tropic acid but also the simultaneously administered about 25×10^2-μmoles/kg. i.v. dose of methyl mandelic acid brought about an apparent complete inhibition of secretion of ethyl mandelic acid over its intravenous dosage range of $2.43–3.03 \times 10^2$ μmoles/kg.

An examination of the data in Fig. 6 indicated that, at the equimolar doses, the apparent glomerular filtration rates of methyl mandelic acid and ethyl mandelic acid are similar in rats. Therefore, it is concluded that the volume of distribution available for these compounds in rats is similar.

Determination of Michaelis–Menten Kinetic Parameters for Renal Tubular Secretion of the Compounds—To determine the V_m and K_m values for methyl mandelic acid and ethyl mandelic acid, the data regarding the apparent initial secretion rates (V) *versus* intravenous doses (D) were obtained by subtracting the apparent initial glomerular filtration rates from the apparent initial excretion rates at the corresponding dosage levels of each of these compounds (Fig. 8). The apparent initial glomerular filtration rates at the intravenous doses above those shown in Fig. 6 were obtained by extrapolation of the straight lines.

As previously shown (2), the plots of $1/V$ *versus* $1/D$ were constructed (Fig. 9) according to the Lineweaver–Burk equation:

$$1/V = K_m/V_m(D) + 1/V_m \qquad \text{(Eq. 2)}$$

The respective values of V_m for these compounds were calculated from the intercepts on the y-axis, and those of K_m were calculated from the slopes of the straight lines. The straight lines were obtained by the method of least squares. The values of V_m and K_m determined for the compounds are listed in Table II. The values of V_m for the two compounds are considered to be comparable by the reasoning offered for considering comparable the values of V_m determined for mandelic acid (18.8 μmoles/kg./min.) and tropic acid (20.8 μmoles/kg./min.) in the previous report (2). Therefore, it is concluded that methyl mandelic acid and ethyl mandelic acid are secreted by the same carrier mechanism present in the renal tubular membrane of rats. It is further concluded from comparing the values of K_m (Table II) for these compounds that the affinity of ethyl mandelic acid for the "carrier" molecules of renal tubular transport is approximately twice that of methyl mandelic acid.

Nature of Inhibition of Secretion of Methyl Mandelic Acid by Tropic Acid—Although methyl mandelic acid, ethyl mandelic acid, and tropic acid may be assumed to exhibit the mutual competitive inhibitory effects on their renal tubular secretion, studies were

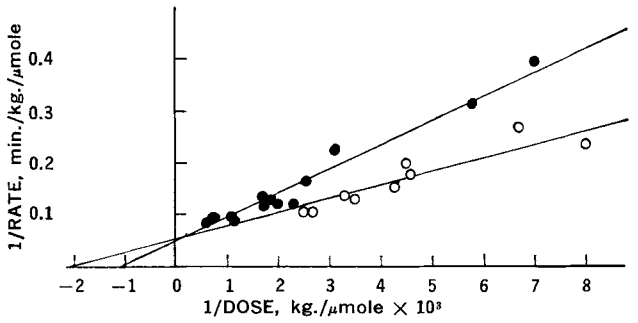

Figure 9—*Lineweaver–Burk plots of the reciprocal of the intravenous dose of* D-(−)-p-*methyl mandelic acid (●) and* D-(−)-p-*ethyl mandelic acid (○) against their reciprocal of the apparent initial secretion rates.*

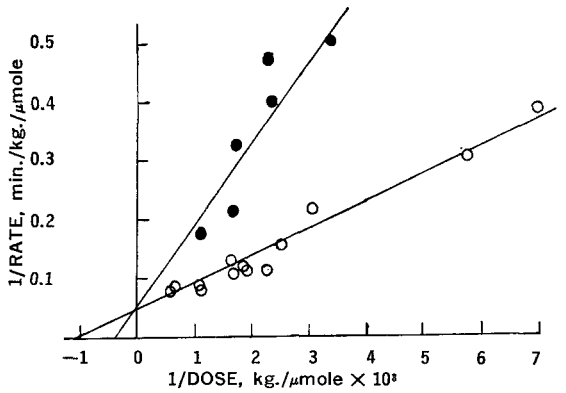

Figure 10—*Lineweaver–Burk plots indicating competitive inhibition of renal tubular secretion of* D-(−)-p-*methyl mandelic acid by* DL-*tropic acid (dose of 21–24 × 10² μmoles/kg. i.v.). Key:* ○, *inhibitor absent; and* ●, *inhibitor present.*

carried out to demonstrate that tropic acid competitively inhibits the secretion of methyl mandelic acid in rats. From the apparent initial excretion rates determined for methyl mandelic acid (2.94–9.03 × 10^2 μmoles/kg.) in the presence of simultaneously administered 21.4–24.1 × 10^2-μmoles/kg. i.v. doses of tropic acid, the apparent initial secretion rates were determined by subtracting from them the apparent glomerular filtration rates at the corresponding doses. The plot of $1/V$ *versus* $1/D$ obtained for the studies is shown in Fig. 10. It becomes evident from the apparent common *y*-intercept in this figure that tropic acid competitively inhibits the secretion of methyl mandelic acid.

Comparison of Michaelis–Menten Kinetic Parameters and Glomerular Filtration Rates of Mandelic Acid and Its Homologs—The values of V_m determined for methyl mandelic acid (21.6 μmoles/kg./min.) and ethyl mandelic acid (18.5 μmoles/kg./min.) in the present study are found to be remarkably similar to those reported (1) for mandelic acid (18.6 μmoles/kg./min.) and tropic acid (20.8 μmoles/kg./min.), thus indicating that the secretion of these compounds occurs in rats *via* the renal tubules by a common "carrier" mechanism. The comparison of the values of K_m for methyl mandelic acid (10.2 × 10^2 μmoles/kg.) and ethyl mandelic acid (4.9 × 10^2 μmoles/kg.) with those of mandelic acid (11.1 × 10^2 μmoles/kg.) and tropic acid (5.2 × 10^2 μmoles/kg.) suggests that the affinities of ethyl mandelic acid and tropic acid for the "carrier" molecules are similar, but these are approximately twice those of methyl mandelic acid and mandelic acid.

Although the affinity of ethyl mandelic acid for the "carrier" molecules is similar to that of tropic acid, the average biological half-life of ethyl mandelic acid (13 min.) observed in the present study is considerably shorter than that reported for tropic acid (22 min.). Also, the average biological half-life of methyl mandelic acid (16 min.) is considerably shorter than that reported for mandelic acid (30 min)., despite the fact that the affinities of these compounds for the "carrier" molecules are similar. These differences in the values of biological half-lives of the compounds can be explained by comparing their apparent glomerular filtration rates, as shown in Fig. 11. At the equimolar doses, the initial glomerular filtration rates obtained for *para*-alkylated mandelic acids (methyl mandelic acid and ethyl mandelic acid) are approximately twice those reported (1) for mandelic acid and its homologs obtained by introducing the methylene group(s) in the side chain of the mandelic acid molecule. This striking difference in the initial glomerular filtration rates of these compounds should be due to the difference in the volume of distribution available in rats for the compounds. Accordingly, the volume of distribution available for methyl mandelic acid and ethyl mandelic acid is almost half that available for mandelic acid and its homologs obtained by adding the methylene group(s) in the side chain of the mandelic acid molecule.

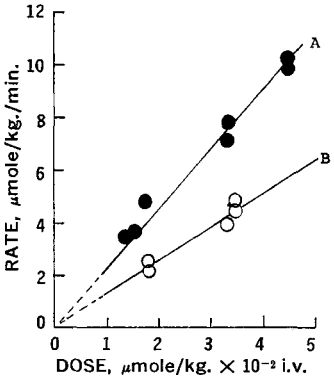

Figure 11—*Comparison of apparent initial glomerular filtration rates observed for* D-(−)-p-*methyl mandelic acid (Curve A) with that reported for* D-(−)-*mandelic acid (Curve B). (Curve A is obtained from Fig. 6 of this paper, and Curve B is adapted from Fig. 4 of* Reference 2.)

Significance of Results—The utilization of the model compounds, methyl mandelic acid and ethyl mandelic acid, in the present studies indicated that the substitution of an alkyl group containing at least two carbon atoms is necessary to increase the affinity of a resulting mandelic acid homolog for the "carrier" molecules of the renal tubular secretion system in rats. This also makes it possible to distinguish a further structural characteristic of the "carrier" molecules in addition to that mentioned in the previous studies (1). From the studies (1) of mandelic acid and its homologs [obtained by introducing methylene group(s) in the side chain of mandelic acid molecule], the possible structural characteristic distinguished was that the hydrophobic region around the cationic site of the carrier molecule probably does not extend uninterruptedly beyond the hydrophobic region represented by two carbon atoms (possibly two methylene groups). The results of the present study seem to support this suggestion, since the *para*-substitution of a methyl group in the mandelic acid molecule did not increase the affinity of the resulting molecule (methyl mandelic acid) for the "carrier," but the *para*-substitution of the ethyl group in the mandelic acid molecule increased the affinity of the resulting molecule (ethyl mandelic acid) for the "carrier." Furthermore, the present findings strongly suggest that the higher affinity of ethyl mandelic acid for the "carrier" molecules than that of mandelic acid or methyl mandelic acid is due to the additional interaction of its end methyl group with the hydrophobic portion that should be present next to the polar group of the "carrier" molecule implied to exist from the previous studies (1).

REFERENCES

(1) E. R. Randinitis, M. Barr, H. C. Wormser, and J. B. Nagwekar, *J. Pharm. Sci.*, **59**, 806(1970).

(2) E. R. Randinitis, M. Barr, and J. B. Nagwekar, *ibid.*, **59**, 813(1970).

(3) B. B. Corsow, R. A. Dodge, S. A. Harris, and J. S. Yeaw, *Org. Syn. Coll., 2nd Ed.*, **1**, 336(1964).

(4) J. J. Klingenberg, *ibid.*, **1**, 110(1964).

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