Kinetics of Urinary Excretion of D-(−)-Mandelic Acid and Its Homologs II: Competitive Inhibitory Effect of D-(−)-Mandelic Acid and DL-Tropic Acid on Their Renal Tubular Secretion in Rats

EDWARD J. RANDINITIS*, MARTIN BARR, and JANARDAN B. NAGWEKAR†

Abstract □ From the pseudo-first-order urinary excretion studies of D-(−)-mandelic acid and DL-tropic acid in rats, data regarding the apparent initial secretion rate *versus* dose (i.v.) are obtained for these compounds and treated according to the Michaelis-Menten kinetics. While the values of maximum apparent initial secretion rate (*Vm*) determined for these compounds are similar, the value of the dose of the substrate required to produce one-half of the maximum apparent initial secretion rate (*Km*) determined for D-(−)-mandelic acid is found to be about twice that determined for DL-tropic acid. Data have been obtained to demonstrate that these compounds competitively inhibit the renal tubular secretion of each other, thereby strongly indicating that these compounds share a common carrier transport system for their secretion. Data obtained in the present studies and those described earlier are utilized to distinguish a certain structural characteristic around the cationic site of the "carrier" molecules of the renal tubular secretion system in rats.

Keyphrases □ D-(−)-Mandelic acid, homologs—urinary excretion □ DL-Tropic acid, D-(−)-mandelic acid—competitive inhibition, urinary excretion □ Structure–excretion rate relationship—D-(−)-mandelic, DL-tropic acids □ Transport system—D-(−)-mandelic acid, DL-tropic acid excretion

The authors (1) have shown that the biological half-lives of DL-tropic acid, DL-phenyllactic acid, D-(−)-benzyllactic acid, and D-(−)-4-hydroxy-4-phenylbutanoic acid, which are homologs of mandelic acid, increased in the presence of D-(−)-mandelic acid in rats. The corresponding decrease in the apparent initial urinary excretion rates of these homologs was also shown to occur in rats in the presence of D-(−)-mandelic acid. The influence of DL-tropic acid on the urinary excretion rate of D-(−)-mandelic acid was also studied. It was demonstrated that the biological half-life of D-(−)-mandelic acid increased and its initial excretion rate decreased in the presence of DL-tropic acid. It was, therefore, suggested that D-(−)-mandelic acid and its previously mentioned homologs share the same transport mechanism for their renal tubular secretion in rats. Since these compounds, which are not significantly metabolized nor bound to the plasma proteins and are excreted entirely in the urine, were found to have different biological half-lives and, at a specific dose, different initial excretion rates, it was further suggested that the common carrier system for renal tubular secretion in rats shows different affinity for these compounds.

Because it is well known that the urinary excretion of most compounds results from a combination of glomerular filtration and tubular secretion, it is conceivable that the compounds used in these studies probably have the same maximum initial secretion rate and initial glomerular filtration rate, if the assumption is correct that these compounds are secreted by the renal tubules of rats by the same carrier mechanism. Therefore, the purposes of the work presented here were to determine the apparent initial glomerular filtration rates and the apparent initial secretion rates of DL-tropic acid and D-(−)-mandelic acid in rats and to analyze the secretion rate data according to Michaelis-Menten kinetics. The additional purpose of the work was to determine if the mutual inhibition caused by these compounds in their renal secretion in rats is competitive or noncompetitive in nature. Since both D-(−)-tropic acid and L-(+)-tropic acid are completely recovered unchanged in the urine of rats and, furthermore, since the biological half-lives of these isomers are found to be similar to each other (1), DL-tropic acid was not resolved into its optical isomers but was used in the racemic form.

EXPERIMENTAL

Materials—D-(−)-Mandelic acid,[1] m.p. 132–133°, $[\alpha]_D^{25}$ −154.2° (c, 1.94, H_2O); and DL-tropic acid,[1] m.p. 118–119°.

Apparatus—The quantitative analysis of mandelic acid and tropic acid appearing in the urine was carried out using an F & M model 810R-19 gas chromatograph by the method described previously (1). A Beckman model 72 pH meter equipped with a combination electrode was used for rat urine pH determinations. The specific rotation of the optically active compound was determined with the aid of J & J Fric model 2706 polarimeter using a sodium lamp as the source of light.

Methodology—The procedure employed for the preparation of the rats and urine collection following the i.v. administration of the compounds was the same as described previously (1). During the course of these studies, approximately 35 Sprague-Dawley male rats weighing between 165 and 215 g. were repeatedly used. None of the rats was used more than five times in these studies; the rest period allowed between the successive use of a rat was at least a week.

To determine the apparent glomerular filtration rate of D-(−)-mandelic acid at dosage levels of 165–330 μmole/kg. (5–10 mg./rat), the appropriate amount of the sodium salt of D-(−)-mandelic acid contained in 0.5 ml. was injected by the i.v. route 15–20 min. after i.p. administration of 15–120 × 10^2 μmole/kg. (50–400 mg./rat) of DL-tropic acid as the sodium salt contained in 5 ml. of water at a pH of 7.0. A similar procedure was followed to determine the apparent glomerular filtration rate of DL-tropic acid at the dosage levels of 150–300 μmole/kg. in the presence of 16–130 × 10^2 μmole/kg. of i.p. administered D-(−)-mandelic acid per rat.

In the studies designed to determine the maximum initial secretion rate (*Vm*) of D-(−)-mandelic acid and its dose required to produce one-half of the maximum initial secretion rate (*Km*), the doses of the sodium salt of D-(−)-mandelic acid in the range of 1.8–21.9 × 10^2 μmole/kg. were dissolved in water (0.5–1.5 ml.) and administered to the rat by the intravenous route. Similar studies were also carried out for DL-tropic acid using the doses of its sodium salt in the range of 1.7–20.0 × 10^2 μmole/kg.

To determine the nature of inhibition of renal tubular secretion of D-(−)-mandelic acid by DL-tropic acid, the initial secretion rates of

[1] Aldrich Chemical Co., Milwaukee, WI 53210

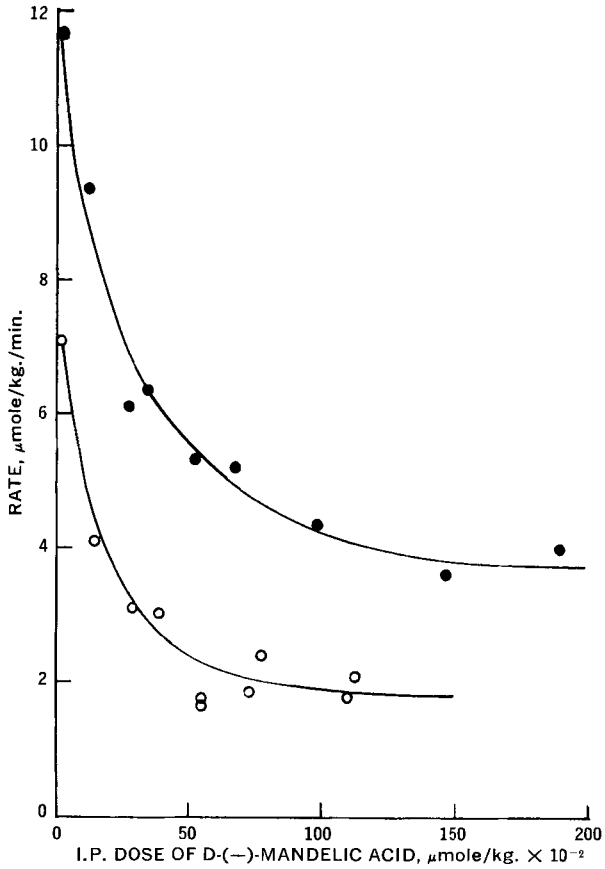

Figure 1—*Determination of the approximate i.p. dose of* D-(−)-*mandelic acid required to obtain a limiting initial excretion rate of* DL-*tropic acid following the administration to rats of its i.v. dose of 170–180 μmole/kg., ○; and 340–350 μmole/kg., ●.*

D-(−)-mandelic acid in the i.v. dosage range of 1.6–8.6 × 10^2 μmole/kg. were determined in the presence of an i.v. dose of 21–26 × 10^2 μmole/kg. (80 mg./rat) DL-tropic acid. Similarly, initial secretion rates for DL-tropic acid in the i.v. dosage range of 4.5–27.4 × 10^2 μmole/kg. were determined in the presence of i.v. doses of 6.0–

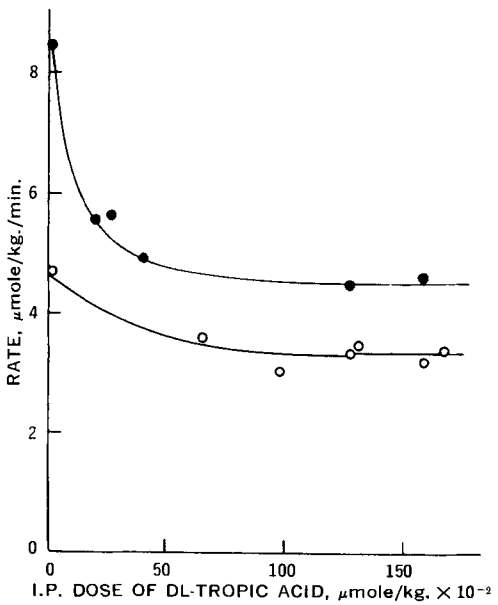

Figure 2—*Determination of the approximate i.p. dose of* DL-*tropic acid required to obtain a limiting initial excretion rate of* D-(−)-*mandelic acid following the administration to rats of its i.v. dose of 170–180 μmole/kg., ○; and 340–350 μmole/kg., ●.*

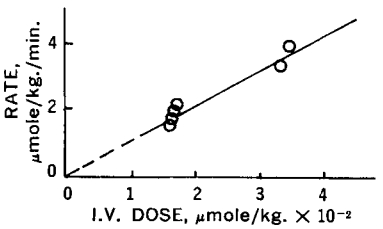

Figure 3—*Relationship between the apparent initial glomerular filtration rate and the i.v. dose for* DL-*tropic acid in rats.*

7.2 × 10^2 μmole/kg. (20 mg./rat), 12–14 × 10^2 μmole/kg. (40 mg./rat), and 24–28 × 10^2 μmole/kg. (80 mg./rat) of D-(−)-mandelic acid. In these studies, DL-tropic acid and D-(−)-mandelic acid were administered by the i.v. route as their sodium salts, and both the substrate and inhibitor compounds were contained in the same i.v. solution.

RESULTS AND DISCUSSION

Determination of the Initial Glomerular Filtration Rate of the Compounds—As mentioned previously, the urinary excretion of most compounds results from a combination of glomerular filtration and renal tubular secretion. Since renal tubular secretion is a saturable process, the reduction can be caused in the excretion rate of a substrate in the presence of an inhibitor that competes for the secretory process. Therefore, it should be possible to inhibit completely the renal tubular secretion of a substrate compound in rats by a simultaneous administration of a large excess of a suitable inhibitor compound. In this event, the initial rate of excretion of the substrate would represent its initial rate of glomerular filtration. It is thus possible to determine the initial rate of glomerular filtration and the initial rate of secretion of a compound at a particular dosage level. Consequently, the initial excretion rates of D-(−)-mandelic acid following i.v. doses of 165–330 μmole/kg. were determined in the presence of increasingly higher doses of DL-tropic acid. The dose of DL-tropic acid, in the range of 12–150 × 10^2 μmole/kg., was administered i.p. about 20 min. prior to the i.v. administration of D-(−)-mandelic acid. In a similar manner, the initial excretion rates of DL-tropic acid following its i.v. doses of 150–300 μmole/kg. were determined in the presence of increasingly higher doses of D-(−)-mandelic acid. The dose of D-(−)-mandelic acid, in the range of 16–130 × 10^2 μmole/kg., was administered i.p. about 20 min. prior to the i.v. administration of DL-tropic acid.

It may be estimated from the uninary excretion data obtained following i.p. administration of 1 × 10^4 μmo'e/kg. of D-(−)-mandelic acid (Fig. 7, *Reference 1*) that, at the 1.65 × 10^4 μmole/kg. dosage level of D-(−)-mandelic acid, the amount of mandelic acid present in the volume of distribution of the rat is approximately 30 times greater than that at the 300 μmole/kg. dosage level of DL-tropic acid and approximately 60 times greater than that at the 150 μmole/kg. dosage level of the acid. At similar large i.p. doses of DL-tropic acid used as the inhibitor of D-(−)-mandelic acid administered at the dosage levels of 165–330 μmole/kg., the amount of DL-tropic acid present in the volume of distribution of the rat would be expected to be in the range described for D-(−)-mandelic acid as the inhibitor. As described previously (1), the apparent initial urinary excretion rates of the compounds were determined by extrapolating the pseudo-first-order plots to zero time. The apparent initial excretion rates obtained from these studies were then plotted against the i.p. dose of the inhibitor as shown in Figs. 1 and 2. It is noted from these plots that an apparently limiting initial excretion rate for each substrate compound is obtained in rats in the presence of 0.9–1.0 × 10^4 μmole/kg. i.p. dose of inhibitor. Since a further decrease in the initial excretion rate was not observed even at higher inhibitor dosage levels, it was evident that at this i.p. dose of inhibitor an apparent saturation of tubular secretion process occurred and the initial excretion rate observed for the substrate was due to the apparent initial glomerular filtration rate. A reasonable linear relationship observed between the apparent initial glomerular filtration rate and the i.v. dose of DL-tropic acid is shown in Fig. 3. It may be seen from the data presented in Fig. 2 that a limiting initial excretion rate (3.4 μmole/kg./min.) obtained for D-(−)-mandelic acid at the dose of 165 μmole/kg. is substantially higher than that expected on the basis of the limiting initial excretion rate (4.5 μmole/kg./min.) obtained at the dose of 330 μmole/kg. Therefore, the initial excretion rate of D-(−)-mandelic acid at the dosage level of 180 μmole/kg. was determined in rats in the presence of a simultaneously administered 3

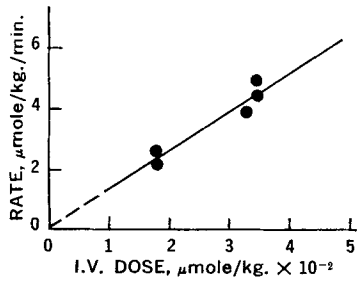

Figure 4—*Relationship between the apparent initial glomerular filtration rate and the i.v. dose for* D-(−)-*mandelic acid in rats.*

$\times 10^3$ μmole/kg. i.v. dose of DL-tropic acid. Both D-(−)-mandelic acid and DL-tropic acid were administered as their sodium salts. The initial excretion rate (2.4 μmole/kg./min.) noted for D-(−)-mandelic acid in this study was of the magnitude expected on the basis of the limiting initial excretion rate obtained at the dose of 330 μmole/kg. Therefore, 2.2 μmole/kg./min. was considered as the apparent glomerular filtration rate at the dose of 165 μmole/kg. of the acid instead of the limiting initial excretion rate noted in Fig. 2. Thus, when these apparent glomerular excretion rates were plotted against the i.v. doses of D-(−)-mandelic acid, a reasonable linear relationship was observed (Fig. 4). These apparent initial glomerular filtration rates observed for D-(−)-mandelic acid are found to be in agreement with those obtained by Khambati and Nagwekar (2) for the acid in the presence of the simultaneously administered 3×10^3 μmole/kg. i.v. dose of DL-tropic acid in rats. Khambati and Nagwekar determined the initial glomerular filtration rates of D-(−)-mandelic acid in the course of determining those for D-(−)-*p*-isopropyl mandelic acid. It seems that the limiting initial excretion rate obtained for D-(−)-mandelic acid at the 165-μmole/kg. dose in Fig. 2 was an experimental artifact. Therefore, the use of the value of the apparent glomerular filtration rate (2.2 μmole/kg./min.) obtained for the acid at the i.v. dosage level of 165 μmole/kg. in the presence of the simultaneously administered 3×10^3 μmole/kg. i.v. dose of DL-tropic acid may be justified in constructing the plot in Fig. 4. It is then theoretically possible to estimate the apparent initial glomerular filtration rate of these acids at higher i.v. doses by the extrapolation of the straight lines shown in Figs. 3 and 4.

Incidentally, since D-(−)-mandelic acid is able to saturate the tubular secretory system upon the administration of an i.p. dose of 1.3×10^4 μmole/kg. in a rat, initial excretion rates reported previously (1) for D-(−)-4-hydroxy-4-phenylbutanoic acid, DL-phenyllactic acid, and D-(−)-benzyllactic acid in the presence of an i.p. dose of 1.3×10^4 μmole/kg. of D-(−)-mandelic acid represent their respective apparent initial glomerular filtration rates, as evidenced by a linear relationship observed in Fig. 5 upon plotting these rates against their i.v. doses. This supports the observation that an i.p. dose of 1.3×10^4 μmole/kg. of D-(−)-mandelic acid serves as an inhibitor of secretion of its homologs and, likewise, an i.p. dose of 1.2×10^4 μmole/kg. of DL-tropic acid serves as an inhibitor of secretion of D-(−)-mandelic acid. The data presented in Fig. 5 also indicate that, in rats, the volume of distribution for all of the compounds used in the study is similar.

Determination of Michaelis-Menten Kinetic Parameters—Since renal tubular secretion represents a saturable system, the transport of molecules through the renal tubular membrane has been described by Michaelis-Menten kinetics (3), and the nature of inhibition of this transport by other compounds has been identified utilizing a Lineweaver-Burk plot technique (4). As in the study of enzyme kinetics, the maximum rate of tubular secretion or transport is generally designated as *Vm* or *Tm*, and the concentration of substrate required to produce one-half of the maximum rate of secretion as *Km*. It is generally expected that for compounds of a homologous series, which are secreted by the same carrier mechanism, the value of *Vm* is the same, but, due to their differences in affinities for the carrier, the value of *Km* for each compound is different. Homologs with a greater affinity for the carrier would be expected to possess a lower value of *Km* (3). In an *in vitro* study of inhibition of uptake of *N'*-methylnicotinamide by kidney slices, Farah *et al.* (4) have applied the Lineweaver-Burk plot technique to demonstrate that organic bases, such as tetraethylammonium, choline, and guanidine, competitively inhibit the uptake of the compound. Cho *et al.* (5) studied the tubular secretion of iodopyracet[2] and *p*-aminohippurate by the renal clearance method in dogs and showed that these compounds competitively inhibit the secretion of each other. To determine the tubular secretion rate, these workers assumed the values of filterability of *p*-aminohippurate and iodopyracet to be 0.92 and 1.00, respectively. The *Vm* value obtained for iodopyracet was 1.6 μmole/kg./min. and that determined for *p*-aminohippurate was 5.0 μmole/kg./min.

[2] Diodrast, Winthrop Laboratories, New York, NY 10016

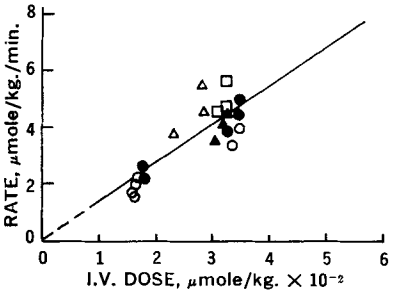

Figure 5—*A plot of the limiting initial excretion rates obtained in rats for* D-(−)-*mandelic acid,* ●; DL-*tropic acid,* ○; D-(−)-4-*hydroxy-4-phenylbutanoic acid,* △; DL-*phenyllactic acid,* □; *and* D-(−)-*benzyllactic acid,* ▲; *following their i.v. administration in the presence of 1.0–1.5* $\times 10^4$ *μmole/kg. of inhibitor. (These data are obtained from Table IV of* Reference 1.)

As applied to secretion kinetics of the present study, the various terms appearing in the following Michaelis-Menten equation (6)

$$v = \frac{Vm\,(S)}{Km + (S)} \qquad \text{(Eq. 1)}$$

are defined as follows. In this equation, v represents the apparent initial secretion rate (μmole/kg./min.), *Vm* the maximum apparent initial secretion rate (μmole/kg./min.), (S) the intravenous dose of the substrate (μmole/kg.), and *Km* the dose of the substrate required to produce one-half of the maximum apparent initial secretion rate (μmole/kg.). From the following Lineweaver-Burk form (7) of Eq. 1,

$$\frac{1}{v} = \frac{Km}{Vm\,(S)} + \frac{1}{Vm} \qquad \text{(Eq. 2)}$$

the values of *Vm* and *Km* are determined from the slope and intercept of the straight line obtained by plotting $1/v$ *versus* $1/(S)$.

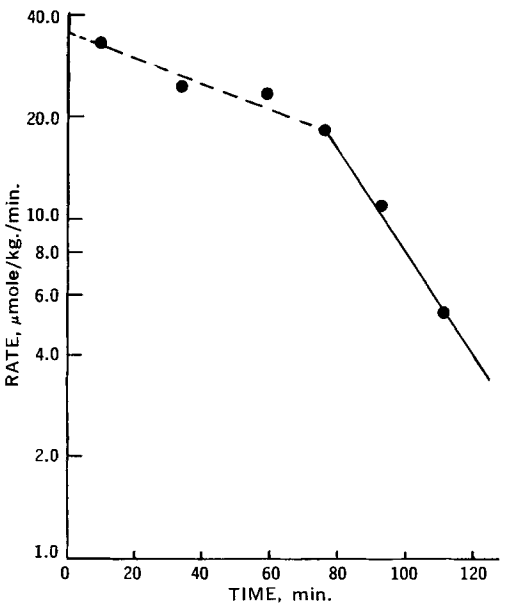

Figure 6—*Urinary excretion data obtained for* D-(−)-*mandelic acid following the i.v. administration of a* 3.37×10^3 *μmole/kg. dose to a rat (indicating the longer biological half-life of the compound during the earlier period being mainly due to an apparent saturation of the secretory process for the compound in the rat).*

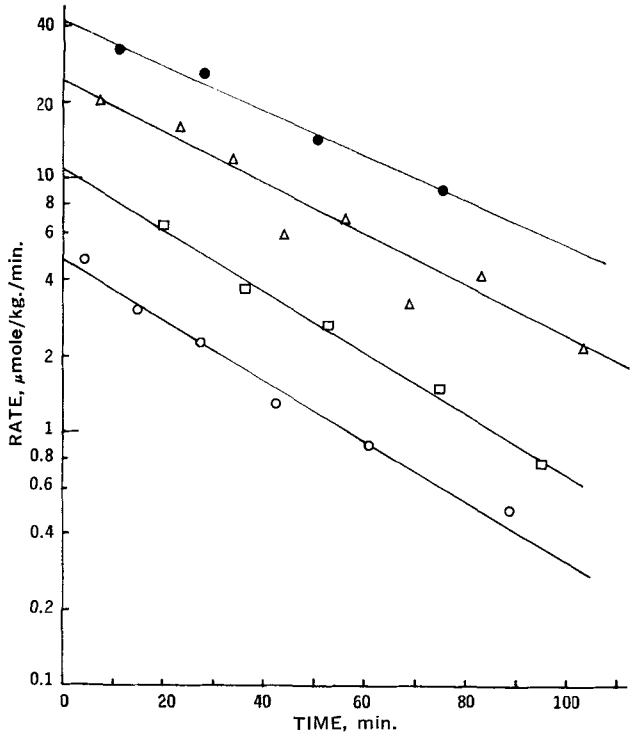

Figure 7—*Apparent first-order urinary excretion data obtained for* D-(−)-*mandelic acid following the i.v. administration of various doses to rats. Key:* ○, *182 μmole/kg.;* □, *533 μmole/kg.;* △, *1169 μmole/kg.; and* ●, *2190 μmole/kg.*

Although the renal clearance method (5) for the *in vivo* determination of Michaelis-Menten kinetic parameters has been used, it was decided to devise a procedure which will make it possible to determine experimentally these parameters without involving a surgical procedure. To apply Michaelis-Menten kinetics to the urinary

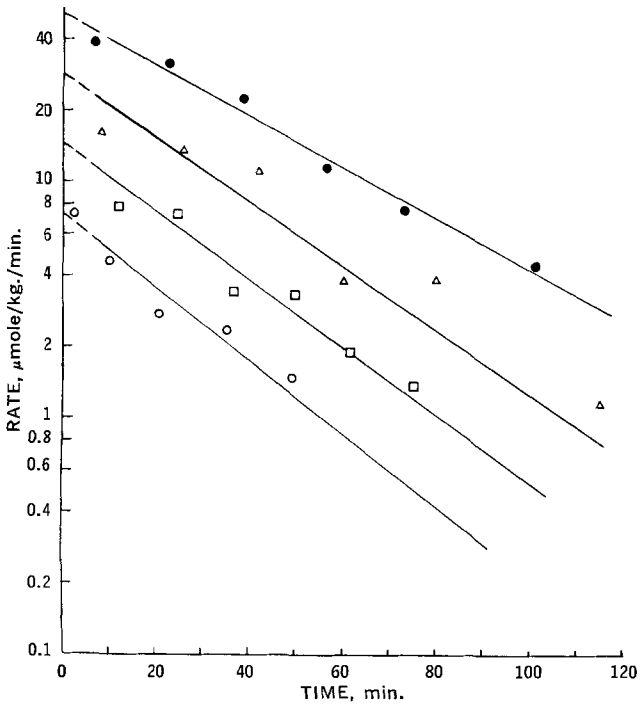

Figure 8—*Apparent first-order urinary excretion data obtained for* DL-*tropic acid following the i.v. administration of various doses to rats. Key:* ○, *167 μmole/kg.;* □, *412 μmole/kg.;* △, *950 μmole/kg.; and* ●, *2110 μmole/kg.*

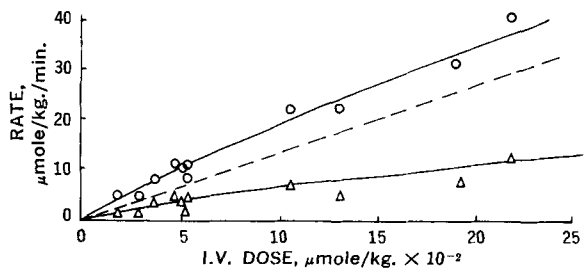

Figure 9—*Apparent initial excretion rates,* ○; *initial glomerular filtration rates,* - - -; *and initial secretion rates,* △; *observed for* D-(−)-*mandelic acid following the i.v. administration of appropriate doses to rats.*

excretion data obtained in the present studies, it was necessary to determine the initial secretion rate of compounds over an appropriate range of dosage levels. The range of doses considered appropriate for a substrate compound in these studies is the range over which not only the urinary excretion of the compounds takes place by a first-order process but also the biological half-life of the substrate remains practically constant. Therefore, the kinetics of urinary excretion of D-(−)-mandelic acid was studied at doses 1.65–4 × 10^2 μmole/kg. It is noted that the biological half-life of D-(−)-mandelic acid remains practically constant (33 min.) over the i.v. dosage range of 1.65–20 × 10^2 μmole/kg. and possibly up to 33 × 10^2 μmole/kg. But at the dosage levels of 33–40 × 10^2 μmole/kg., a slower excretion rate (increased biological half-life) was observed for an hour following the i.v. administration of D-(−)-mandelic acid, which may mainly be due to an apparent saturation of the secretory process of the compound (Fig. 6). Therefore, the dosage range of this acid considered appropriate in this study was 1.65–20 × 10^2 μmole/kg. The results obtained for DL-tropic acid were found to be similar to those obtained for D-(−)-mandelic acid and, therefore, the dosage range of this acid considered suitable was 1.5–20 × 10^2 μmole/kg. The data obtained for the urinary excretion of D-(−)-mandelic acid following the i.v. administration of various doses are presented in Fig. 7. Similarly, the data obtained for the urinary excretion of DL-tropic acid are presented in Fig. 8.

The apparent initial renal tubular secretion rates were determined from the total initial excretion rates at each i.v. dosage level of DL-tropic acid, as well as D-(−)-mandelic acid, by subtracting the apparent initial glomerular filtration rates noted for these compounds from Figs. 3 and 4 at the corresponding dosage levels. The plot of the observed apparent initial excretion rate *versus* dose (i.v.) and the plot of the initial secretion rate *versus* dose (i.v.) are also constructed for these compounds, as shown in Figs. 9 and 10. The extrapolated lines due to the apparent initial glomerular filtration rate *versus* dose for these compounds are included in Figs. 9 and 10. The curvilinear plots obtained for the initial excretion rate data are in accordance with the expectation for compounds which are excreted by both glomerular filtration and tubular secretion (8).

To determine the Michaelis-Menten kinetic parameters, the reciprocal of the initial secretion rate was plotted against the reciprocal of the i.v. dose for both D-(−)-mandelic acid and DL-tropic acid (Fig. 11). From the slope of the straight line obtained by the method

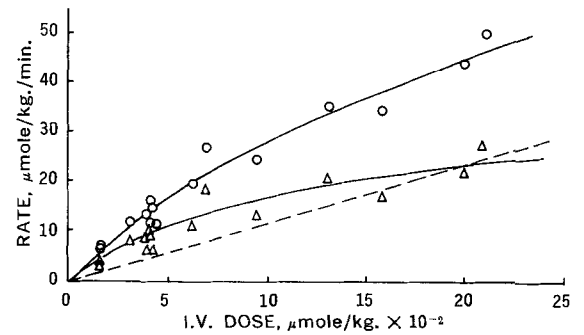

Figure 10—*Apparent initial excretion rates,* ○; *initial glomerular filtration rates,* - - -; *and initial secretion rates,* △; *observed for* DL-*tropic acid following the i.v. administration of appropriate doses to rats.*

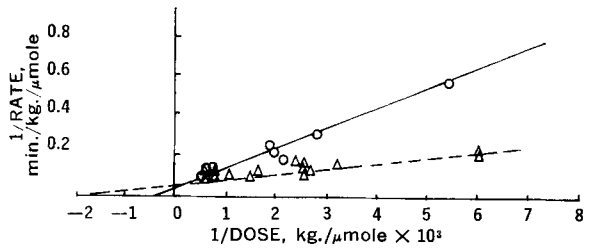

Figure 11—*Lineweaver-Burk plots of the reciprocal of the i.v. dose of* D-(−)-*mandelic acid,* ○; *and* DL-*tropic acid,* △; *against their corresponding reciprocal of the apparent initial secretion rates.*

of least squares and from the *y*-intercept obtained by extrapolation of the straight line, the respective values of *Km* and *Vm* were determined for D-(−)-mandelic acid and for DL-tropic acid. These values are listed in Table I. Values for the maximum secretion rate (*Vm*) obtained for *p*-aminohippurate in rats by the renal clearance method have been reported by several workers (9–11). For the sake of comparison, when these *Vm* values are converted to units of μmole/kg./min., they are found to be 9.27, 14.93, and 18.02 μmole/kg./min. Therefore, in light of such a wide variation observed by other workers in the *Vm* values for *p*-aminohippurate, the *Vm* value of 18.8 μmole/kg./min. obtained for D-(−)-mandelic acid may be considered comparable to the *Vm* value of 20.8 μmole/kg./min. obtained for DL-tropic acid, thus strongly suggesting that the two acids are secreted by the same carrier mechanism present in the renal tubule of the rat. The values of *Km* obtained in these studies for DL-tropic acid (5.2×10^2 μmole/kg.) and D-(−)-mandelic acid (11.1×10^2 μmole/kg.) strongly indicate that the affinity of DL-tropic acid for the renal tubular transport carrier is about twice that of D-(−)-mandelic acid.

Determination of the Nature of Inhibition—To determine whether the mutual inhibition observed for DL-tropic acid and D-(−)-mandelic acid is competitive or noncompetitive in nature, the apparent initial secretion rates of DL-tropic acid at various intravenous dosage levels were determined in the presence of a fixed dose of D-(−)-mandelic acid, and those of D-(−)-mandelic acid at various intravenous dosage levels were determined in the presence of a fixed dose of DL-tropic acid, and analyzed by the Lineweaver-Burk plot method. Accordingly, following the administration of 1.65–26 $\times 10^2$ μmole/kg. doses, apparent initial excretion rates of D-(−)-mandelic acid were determined in the presence of 21–26 $\times 10^2$ μmole/kg. (80 mg./rat) of DL-tropic acid. Similarly, initial excretion rates for DL-tropic acid at dosage levels of 3–24 $\times 10^2$ μmoles/kg. were determined in the presence of 6.0–7.2 $\times 10^2$ μmole/kg. (20 mg./rat), 12–14 $\times 10^2$ μmole/kg. (40 mg./rat), and 24–28 $\times 10^2$ μmole/kg. (80 mg./rat) doses of D-(−)-mandelic acid. From these data, initial secretion rates for the substrates were calculated in the manner described earlier. The reciprocals of the substrate dose were then plotted against the reciprocals of the initial excretion rate of the substrate, as shown in Figs. 12 and 13. The straight-line plots shown for the inhibitory studies involving 20, 40, and 80 mg. of inhibitor were constructed by visual inspection. It becomes evident from the apparent common *y*-intercept in Figs. 12 and 13 that these acids competitively inhibit the renal tubular secretion of each other in rats.

Possible Implications of the Results Obtained in the Studies—Although the *Vm* and *Km* values have not been determined for DL-phenyllactic acid, D-(−)-benzyllactic acid, and D-(−)-4-hydroxy-4-phenylbutanoic acid, it is observed from the inhibitory effect of D-(−)-mandelic acid on the urinary excretion of these compounds that their apparent glomerular filtration rates are similar to those of D-(−)-mandelic acid and DL-tropic acid (Fig. 5). Therefore, it is not unreasonable to conclude that there exists a common carrier system in the renal tubule membrane of rats for the transport of the acids employed in the present study.

Table I—The Michaelis-Menten Kinetic Parameters of Secretion for D-(−)-Mandelic Acid and DL-Tropic Acid in Rats

Compound	Apparent *Vm*,[a] μmole/kg./min.	Apparent *Km*,[a] μmole/kg. $\times 10^{-2}$
D-(−)-Mandelic acid	18.8 ± 3.1[b]	11.1 ± 2.5[b]
DL-Tropic acid	20.8 ± 2.5	5.2 ± 1.8

[a] The values of the apparent *Vm* obtained for the compounds are not significantly different from each other, while the values of the apparent *Km* determined for the compounds are significantly different from each other ($p < 0.01$). [b] Standard deviation for each compound was determined from 11–15 rat studies described in Fig. 11.

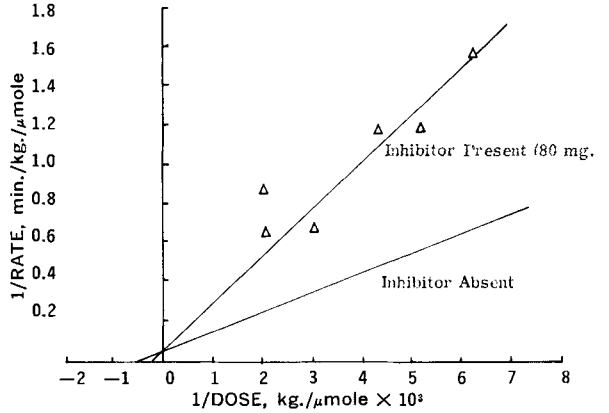

Figure 12—*Lineweaver-Burk plots indicating competitive inhibition of secretion of* D-(−)-*mandelic acid by* DL-*tropic acid in the i.v. dose of 80 mg./rat (21–26 $\times 10^2$ μmole/kg.).*

The additional purpose of this project was to determine the effect of the incorporation of methylene groups in the side chain of the mandelic acid molecules on the rate of secretion, and thereby to gain some insight into the possible chemical nature of the carrier molecule in the vicinity of its positively charged primary binding site. Based on the data obtained in these studies, it seems possible to distinguish certain characteristics of the carrier molecule.

It has been illustrated in the present studies that the incorporation of methylene group(s) in the side chain of the mandelic acid molecule results in a significant decrease (1) in the biological half-life of the compounds due to an increase in the rate of renal tubular secretion. DL-Tropic acid ($t_{1/2}$, 23 ± 4 min.) and DL-phenyllactic acid ($t_{1/2}$, 20 ± 3 min.), each possessing one more methylene group than does mandelic acid, are found to have significantly shorter biological half-lives than that of D-(−)-mandelic acid ($t_{1/2}$, 30 ± 4 min.). Although the D-(−)-4-hydroxy-4-phenylbutanoic acid and D-(−)-benzyllactic acid molecules contain two more methylene groups than does the molecule of mandelic acid, the biological half-life of D-(−)-4-hydroxy-4-phenylbutanoic acid ($t_{1/2}$, 16 ± 2 min.) is significantly shorter than that observed for D-(−)-benzyllactic acid ($t_{1/2}$, 21 ± 3). It was also observed that the biological half-lives obtained for DL-phenyllactic acid and D-(−)-benzyllactic acid are practically the same. These observations indicate that the presence of methylene groups next to the carboxyl group contribute significantly to the affinity of the mandelic acid homolog to the carrier molecule. Since the primary interaction of the substrate molecule with the carrier molecule is expected to be due to electrostatic forces, the further affinity of the homologs of mandelic acid may be attributed to the secondary interaction (12–14) between the hydrophobic groups of the carrier molecule and the homolog. Therefore, it is suggested that the hydrophobic groups, such as methylene groups, should be pres-

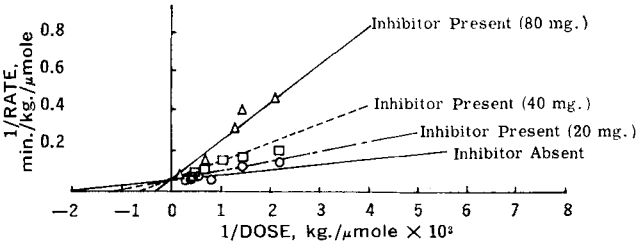

Figure 13—*Lineweaver-Burk plots indicating competitive inhibition of secretion of* DL-*tropic acid by* D-(−)-*mandelic acid in the i.v. doses of* △, *80 mg./rat (36–44 $\times 10^2$ μmole/kg.);* □, *40 mg./rat (12–14 $\times 10^2$ μmole/kg.); and* ○, *20 mg./rat (6.0–7.2 $\times 10^2$ μmole/kg.).*

ent next to the positively charged group or site on the carrier molecule.

An examination of the chemical structures of D-(−)-benzyllactic acid and D-(−)-4-hydroxy-4-phenylbutanoic acid (1) may shed some light on the extent of the hydrophobic group in the vicinity of the cationic site of the carrier molecule. Both of these compounds have the same molecular weight, chemical composition, and absolute configuration. The only respect in which these molecules differ from each other is the position of the methylene groups in relation to the carboxyl group. In a D-(−)-4-hydroxy-4-phenylbutanoic acid molecule, the two methylene groups are present next to the carboxyl group; in a D-(−)-benzyllactic acid molecule, the two methylene groups are separated from the carboxyl group by the carbon containing the hydroxyl group. Since the biological half-life for D-(−)-4-hydroxy-4-phenylbutanoic acid is significantly shorter than that for D-(−)-benzyllactic acid, it suggests that the hydrophobic region in close proximity to the cationic groups of the carrier molecule probably does not extend uninterrupted beyond the hydrophobic region present in close proximity to the carboxyl group of the D-(−)-4-hydroxy-4-phenylbutanoic acid molecule. This inference seems to be supported by the fact that the biological half-lives of D-(−)-benzyllactic acid and DL-phenyllactic acid are practically the same, in spite of the facts that the benzyllactic acid molecule contains one more methylene group than does the phenyllactic acid molecule and the position of the hydroxyl group in relation to the carboxyl group in these molecules is the same.

The comparison of the biological half-life data obtained for D-(−)-mandelic acid, DL-tropic acid, and DL-phenyllactic acid also seems to support this view regarding the hydrophobic region in close proximity to the positively charged site on the carrier molecule. Perhaps the use of 5-hydroxy-5-phenylpentanoic acid would have offered some additional information in this regard, but its extensive metabolism by rats precluded its use in the present studies. However, studies similar to those described here are being carried out with other homologs of D-(−)-mandelic acid to distinguish further characteristics of the carrier molecule.

SUMMARY AND CONCLUSIONS

Using DL-tropic acid and D-(−)-mandelic acid as the mutual competitive inhibitors of their renal tubular secretion, urinary excretion kinetic studies were carried out in rats to determine their initial glomerular filtration rates at various doses without involving a surgical procedure. The initial rate–i.v. dose profiles were obtained for urinary excretion, renal secretion, and glomerular filtration of these compounds.

The Michaelis-Menten kinetic parameters (*Vm* and *Km*) of secretion were determined for each of the two compounds. The values of *Vm* determined for these compounds are found to be similar, but the value of *Km* obtained for D-(−)-mandelic acid is approximately twice that obtained for DL-tropic acid, indicating that the affinity of the former for the renal tubular transport carrier is about one-half that of the latter.

Based on the initial glomerular filtration rate data obtained for D-(−)-mandelic acid and DL-tropic acid in this study and those obtained for DL-phenyllactic acid, D-(−)-benzyllactic acid, and D-(−)-4-hydroxy-4-phenylbutanoic acid in the previous study, it was concluded that the volume of distribution of D-(−)-mandelic acid and its above homologs is similar in rats.

The results of these studies have been utilized to attempt to distinguish certain structural characteristics around the positively charged site of the carrier molecules for renal tubular secretion in rats.

REFERENCES

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

(2) M. Khambati and J. B. Nagwekar, to be published.

(3) H. N. Christensen, "Biological Transport," W. A. Benjamin, New York, N. Y., 1962, pp. 45–51.

(4) A. Farah, M. Frazer, and E. Porter, *J. Pharmacol. Exp. Ther.*, **126,** 202(1959).

(5) K. C. Cho, J. H. Kim, S. K. Hong, and W. C. Lee, *Yonsei Med. J.*, **1,** 25(1960).

(6) L. Michaelis and M. L. Menten, *Biochem. Z.*, **49,** 333(1913).

(7) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56,** 658(1934).

(8) J. G. Wagner, *Drug Intel.*, **2,** 95(1968).

(9) S. M. Friedman, J. R. Polley, and C. L. Friedman, *Amer. J. Physiol.*, **150,** 340(1947).

(10) A. C. Corcoran, G. Musson, R. Reuting, and I. H. Page, *ibid.*, **154,** 170(1948).

(11) A. C. Corcoran and I. H. Page, *Fed. Proc.*, **6,** 91(1947).

(12) A. Despopoulos, *J. Theor. Biol.*, **8,** 163(1965).

(13) I. M. Weiner and G. H. Mudge, *Amer. J. Med.*, **36,** 743 (1964).

(14) A. Essig and J. V. Taggert, *Amer. J. Physiol.*, **199,** 509 (1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 4, 1969, from the *College of Pharmacy, Wayne State University, Detroit, MI 48202*

Accepted for publication February 4, 1970.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

Abstracted in part from a dissertation submitted by E. J. Randinitis to the Graduate School, Wayne State University, Detroit, in partial fulfillment of the Doctor of Philosophy degree requirements.

* Present address, Product Development Research Laboratories, Parke, Davis & Co., Detroit, MI 48232. American Foundation for Pharmaceutical Education Fellow, 1967–1968.

† To whom all inquiries should be directed.