Kinetics of Urinary Excretion of D-(−)-Mandelic Acid and Its Homologs I: Mutual Inhibitory Effect of D-(−)-Mandelic Acid and Its Certain Homologs on Their Renal Tubular Secretion in Rats

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Abstract □ It has been shown from the apparent first-order urinary excretion studies of D-(−)-mandelic acid and certain of its homologs, DL-tropic acid, D-(−)-4-hydroxy-4-phenylbutanoic acid, DL-phenyllactic acid, and D-(−)-benzyllactic acid, that the biological half-lives of the homologs are significantly shorter than that of D-(−)-mandelic acid in rats. Since these compounds, which differ from each other in their content of methylene groups around the carboxyl group, exhibit negligible metabolism and protein binding, low pKa values (3.3–4.7), and low lipid solubility at the physiological pH, and are primarily recovered in the urine in the intact form, they are utilized in these studies as model compounds to study the effect of hydrophobic group(s) on the rate of secretion. These compounds are shown to exert a mutual inhibitory effect on their renal tubular secretion, indicating a common "carrier" mechanism for their secretion. The utilization of these compounds indicated that the addition of methylene group(s) in the vicinity of the carboxyl group of the mandelic acid molecule increased its affinity for the carrier molecules of the renal tubular secretion system in rats.

Keyphrases □ D-(−)-Mandelic acid and homologs—mutual inhibition of urinary excretion □ Urinary excretion—D-(−)-mandelic acid and homologs □ Excretion rates, apparent—D-(−)-mandelic acid and homologs □ ORD—identity □ Polarimetry—identity □ GLC—analysis

Although considerable work has been reported showing the effect of variation of hydrophobic groups within a weakly acidic drug molecule on its rate of transport through kidney slices (1–3), little such work has been carried out *in vivo*. Furthermore, in *in vivo* work, the determination of the effect of hydrophobic variations in the molecule on the rate of urinary excretion and kidney tubular secretion has been shown to be complicated due to the differences of the homologs in their extent of binding to plasma proteins, pKa values, and partition coefficients between a lipid phase and water. The objectives of such studies were to investigate the physicochemical nature of the transport system involved in the kidney tubular secretion of drugs and other chemicals and to gain some insight into the structural specificity of a drug molecule for the transport system. The compounds commonly employed in such studies are *p*-aminohippurate (4), phenylbutazone (5), *p*-aminosalicylic acid (6), salicylic acid (2), and chlorothiazide (7).

From the studies of renal excretion of phenylbutazone analogs (5) and sulfonamide analogs (8), it has been shown that the rate of excretion of most of these compounds is inversely related to their pKa values at the normal slightly acidic urine pH. However, in their inhibitory studies of hippurate transport by phenylbutazone analogs in the kidney slices of dogs and rabbits, Despopoulos *et al.* (9) found a lack of a clearcut relationship between the inhibitory capacity of the phenylbutazone analogs and their pKa values and lipid solubility. Despopoulos and Callahan (10) observed that the reabsorption of certain sulfonamides with pKa values greater than the normal urine pH of 6 to 7 occurs from the renal tubules, and it makes difficult the interpretation of data to correlate the chemical affinity of drug analogs to the renal transport system. This problem becomes further complicated if a drug is largely metabolized and the metabolites interfere with the secretion of the parent compound, as observed with substituted benzoic acids (11).

Rammelkamp and Bradley (12) and Beyer *et al.* (13) were among the first to demonstrate that iodopyracet[1] and *p*-aminohippurate cause an increase in the blood levels of penicillin due to the competition of the inhibitors with penicillin for the carrier of secretory process in the renal tubular membrane. Similar inhibitory effects of probenecid on the urinary excretion of the optical isomers of mandelic acid have been demonstrated in humans by Nagwekar and Kostenbauder (14). Kamienny *et al.* (15) have also shown that, in humans, sulfadiazine, sulfamerazine, sulfamethazine, and (−)-mandelic acid probably share the same renal tubular transport mechanism for secretion. Various possible mechanisms suggested for the inhibition of transport of compounds through renal tubular membranes have been shown to depend on the chemical nature of the substrate and the inhibitor (16). The type of inhibition which is widely studied for the transport of substances across the renal tubular membrane is that in which the substrate and inhibitor probably share the same transport carrier.

The purposes of this project were to study the kinetics of urinary excretion of a model acidic compound and its homologs, which differ from each other in the content of their hydrophobic groups, such as methylene groups, and to determine their biological half-lives and initial excretion rates in the absence and presence of each other in rats. If such compounds are secreted by the same pathway, it may then be expected that they would mutually inhibit the secretion of each other. For reasons described later, D-(−)-mandelic acid is considered as a model compound and is used in the present studies. The compounds used in these studies are listed in Table III. The differences in the initial urinary excretion rates or the biological half-lives that these compounds show are attributed to the differences in the hydrophobic interactions which take place between the compounds and a carrier or carrier system. As described in the next paper

[1] Diodrast, Winthrop Laboratories, New York, N. Y.

in this series (17), an additional objective of this project was to gain some insight into the structural and chemical specificity that the carrier transport system of the renal tubule shows toward anionic drugs by studying the Michaelis-Menten-type kinetics of secretion of these compounds.

EXPERIMENTAL

Materials—D-(−)-Mandelic acid,[2] m.p. 132–133°, $[\alpha]_{25}^{D}$ − 154.2° (c, 1.94, H_2O); DL-tropic acid,[2] m.p. 118-119°; D-(−)-tropic acid, m.p. 123-124°, $[\alpha]_{25}^{D}$ −75° (c, 1.60, H_2O); and L-(+)-tropic acid, m.p. 126–127°, $[\alpha]_{25}^{D}$ + 77.4° (c, 1.94, H_2O), were obtained by the resolution of DL-tropic acid by the method of King (18); DL-phenyllactic acid, m.p. 96–97°; L-(−)-phenyllactic acid, m.p. 122–123°, $[\alpha]_{25}^{D}$ −20.0° (c, 1.89, H_2O); and D-(+)-phenyllactic acid, m.p. 121–122°, $[\alpha]_{25}^{D}$ + 21.2° (c, 2.05, H_2O), were synthesized by the method of Eiduson *et al.* (19) from the corresponding isomers of phenylalanine. D-(−)-Benzyllactic acid, m.p. 113–115°, $[\alpha]_{25}^{D}$ −10.0° (c, 1.50, ethanol), and the sodium salt of D-(−)-4-hydroxy-4-phenylbutanoic acid, $[\alpha]_{25}^{D}$ −12.9° (c, 1.55, H_2O), were obtained on resolving (20, 21) the corresponding racemic acids synthesized by the known procedures (22).

Apparatus—The compounds appearing in the urine of rats were quantitatively analyzed with the aid of an F & M model 810R-19 gas chromatograph equipped with a hydrogen-flame detector. The column employed was a 1.21-m. (4-ft.) long and 0.63-cm. (0.25-in.) o.d. copper tube packed with 80–100 mesh diatomite (Diatoport S) coated with 5% ethylene glycol succinate. Helium was employed as the carrier gas. A Beckman model 72 pH meter equipped with a combination electrode was used for the pH determinations. The specific rotation of the optically active compounds were determined at 25° with the aid of a J & J Fric model 2706 polarimeter using a sodium lamp as the source of light. The optical rotatory dispersion (ORD) curves of the compounds were obtained with the aid of a Cary model 60 recording spectropolarimeter.

Methodology—During the course of these studies, about 30 Sprague-Dawley male rats weighing between 165 and 210 g. were repeatedly used. None of the rats was used more than five times in these studies, and the rest period allowed between the successive use of a rat was at least 1 week. Food was withheld from the animals for 12–18 hr. prior to the administration of the compounds and during urine collection; water was allowed *ad libitum* throughout this period. To induce prompt urination, 5 ml. of water was administered intraperitoneally to each rat 15–20 min. prior to the intravenous administration of the compounds.

The compounds were administered in the dosage range of 150–700 μmole/kg. (5–20 mg./rat) by the intravenous route *via* the tail vein. The compounds were administered as aqueous isotonic solutions of their sodium salts, prepared by the addition of an equivalent amount of sodium hydroxide to an aqueous solution of the acid. In inhibitory urinary excretion studies involving equivalent doses of each compound, an isotonic solution was prepared containing the appropriate dose of the sodium salt of each of the compounds and administered by the i.v. route to the rats. However, in the inhibitory studies involving a larger dose of the inhibitor, the i.p. administration of $1.0–1.7 \times 10^4$ μmole/kg. (300–500 mg./rat) dose of inhibitor was found necessary. A solution containing an appropriate dose of the sodium salt of the inhibitor dissolved in 5 ml. of water was prepared and administered by the i.p. route 15–20 min. prior to the i.v. administration of the substrate compound.

Each rat was placed into a urine-collection cage immediately after the administration of the substrate compound. The urine-collection cage consisted of a 20.32-cm. (8-in.) plastic funnel, two chrome-plated wire baskets, and a 50-ml. graduated cylinder as shown in Fig. 1. A fine wire-mesh screen was fitted at the inner opening of the funnel to prevent rat feces from contaminating the urine. However, the rats rarely defecated during the first 3 hr. of urine collection, during which time most of the compound was recovered. Also, it was demonstrated in the preliminary studies that the excretion of the compounds in the feces was negligible. An animal drinking tube was forced through the wire basket to allow the animal water during the study.

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

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

Compound	Oven Temp.[a]	He Flow Rate, ml./min.	Retention Time, min.
Methyl mandelate	165°	60	5.8
Methyl tropate	180°	40	8.2
Methyl phenyllactate	180°	40	5.4
Methyl benzyllactate	180°	40	7.4
Phenylbutyrolactone	175°	60	17.0

[a] The injection port and detector temperatures were maintained at 210°.

After the administration of the compound, the rat was observed for 2 hr. in which four to seven urine samples were carefully collected at intervals of 10–30 min. After each passage of urine, the genitalia of the rat were washed with a stream of water from a wash bottle, and the rat was transferred to another urine-collection cage. The cage was thoroughly washed with water, and the urine and washings were brought up to a suitable volume with water. The subsequent urine samples collected over a period of 2–24 hr. were pooled into one sample prior to analysis. The urine samples were analyzed on the same day of collection for the compounds.

Analysis of the Compounds—The urine samples were quantitatively analyzed for intact compounds appearing in the urine by a gas chromatographic method described by Kamienny *et al.* (15) after converting the acids to their methyl esters upon treating with diazomethane. This procedure was employed for the analysis of all the compounds except 4-hydroxy-4-phenylbutanoic acid, which was converted to the corresponding lactone by the addition of enough 5 *N* hydrochloric acid solution to adjust the pH of the urine sample to approximately 2 prior to its ether extraction and gas chromatographic analysis. This was necessary because 4-hydroxy-4-phenylbutanoic acid readily forms a lactone in the strongly acidic medium, and the lactone thus formed can be quantitatively analyzed by gas chromatography. The lactone is readily reconverted to the 4-hydroxy-4-phenylbutanoate ion at pH 6 and above, which usually is the pH range of rat urine. The conditions employed for the gas chromatographic analysis and the retention times observed for the methyl esters of the compounds are described in Table I.

Dissociation Constants, Apparent Partition Coefficients, and Binding of Compounds to Whole Blood (Rat)—The dissociation constants of the compounds were determined using a titrimetric procedure. A 20-mg. quantity of each acid dissolved in 10 ml. of

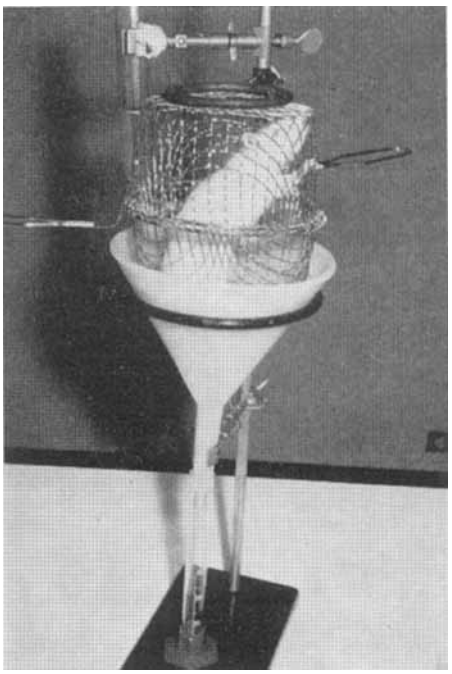

Figure 1—*Urine collection cage employed in the studies.*

Table II—Values of pKa and Partition Coefficient Determined for the Compounds

Compound[a]	pKa (Observed)	Apparent Partition Coefficient $C_{chloroform}/C_{water}$	Apparent Partition Coefficient C_{ether}/C_{water}
D-(−)-Mandelic acid	3.35 (3.36)[b]	[c]	[d]
DL-Tropic acid	4.20 (4.12)[b]	[c]	[d]
DL-Phenyllactic acid	3.80	[c]	[d]
D-(−)-Benzyllactic acid	3.85	<0.01	<0.01
D-(−)-4-Hydroxy-4-phenylbutanoic acid	4.70	0.32	0.35

[a] The assignment of the absolute configuration of the compounds was based on their optical rotatory dispersion curves as explained in the text. [b] Values reported in the literature. [c] The compound was absent in the chloroform layer. [d] The compound was absent in the ether layer.

water was titrated with 0.01 *N* sodium hydroxide solution; the pKa, which is equivalent to the pH at one-half neutralization, was determined.

An apparent partition coefficient was determined between a phosphate buffer at pH 7.0 and ether or chloroform representing the lipid phase. In each case, 5 mg. of the acid was dissolved in 10 ml. of the buffer and shaken with an equal volume of either chloroform or ether. Each phase was analyzed for acid content by the gas chromatographic procedure.

The studies to determine the binding of D-(−)-mandelic acid and each of its homologs by whole blood (rat) were carried out by the equilibrium dialysis procedure. Whole blood (rat) was obtained by decapitation, collecting the blood in a 150-ml. beaker previously coated with 2 ml. of heparin sodium.[3] Four milliliters of freshly collected whole blood was placed into a Visking dialysis bag, sealed, and placed into a 4–5-dr. (15–20 ml.) bottle with a screw cap. Then, 4.0 ml. of an isotonic phosphate buffered (pH 7.4) solution, containing 1.0 mg. of D-(−)-mandelic acid or its homolog, was placed into the bottle; the bottle was sealed and shaken at 37 ± 0.5° for 6 hr. during which the equilibrium for the system was attained. The bottles were removed from the shaker and immediately the liquid outside of the bag was transferred to a 10-ml. beaker and allowed to attain room temperature. These solutions were then analyzed for mandelic acid or its homolog by the gas chromatographic method. Control studies were carried out in which 4 ml. of the phosphate buffer solution was used in place of 4 ml. of blood. Neither D-(−)-mandelic acid nor any of its homologs used in the present study was found to bind to the dialysis membrane.

RESULTS AND DISCUSSION

Selection of a Model Compound and Its Homologs—Recent studies dealing with the transport of ionic and polar substances through biological membranes have pointed to the existence of a carrier system in the membrane which facilitates such transport. According to current theory, there exists in the membrane separate carrier systems responsible for the transport of organic anions and cations through the renal tubular membrane (2, 11, 16, 23, 24). Evidence for the presence of a common carrier system for the active transport of organic anions has been reported (1, 11). Although the carrier system has not yet been isolated, its constituents are suggested to be protein and/or phospholipid in nature. It is also proposed that while the primary linkage between the carrier and substrate compound to be transported occurs through ionic bonds, the secondary linkages result from hydrophobic bonding between the nonpolar portions of the carrier and substrate molecules or from hydrogen bonding between the appropriate portions of the substrate and carrier molecules (1–3). Such secondary bonding forces are expected to become important in relating the affinity of the molecules to the carrier. Therefore, it is considered of interest to determine the initial rate of secretion of compounds which vary slightly in their content of methylene groups around their anionic sites and to relate the differences in their initial secretion rates to the differences in their hydrophobicity in the molecule.

[3] Heparin sodium USP, 1000 units/ml., Testagar and Co., Inc., Detroit, Mich.

The selection of a model compound and its homologs was based on several important properties considered desirable for this study. Among these properties, the most important was that the compounds should be involved in the renal tubular secretory process and be recovered primarily in the urine in the intact form, since this will preclude the possible inhibitory effect of the metabolites on the secretion of the parent compound. The compounds should possess sufficiently low pKa values, so that they will remain in the urine mainly in the ionized form and their passive reabsorption from the renal distal tubule will be minimized. The partition coefficient of the compounds between a lipid phase and water (pH 6–8) should also be as low as possible to prevent their tubular reabsorption. The compounds should exhibit little or no tendency to bind to plasma proteins, since the urinary excretion rate depends on the concentration of the free form of the compound in the blood.

The literature survey indicated that D-(−)-mandelic acid (14, 15) is not metabolized significantly by humans and that DL-tropic acid (25) is not metabolized by rats. Therefore, appropriate preliminary studies carried out in rats indicated that D-(−)-mandelic acid and its homologs (listed in Table II) possess most of the properties described. The recovery of each compound in the unchanged form in the rat urine was found to be 90–100% of the administered i.v. dose. These compounds differ from mandelic acid by the addition of methylene groups in specified positions, in some cases (phenyllactic acid and benzyllactic acid) maintaining the hydroxyl group adjacent to the carboxyl groups as in the mandelic acid molecule, and in others (tropic acid and 4-hydroxy-4-phenylbutanoic acid) separating these groups by the additional methylene groups.

The overall metabolism studies of 3-hydroxy-3-phenylpropanoic, 5-hydroxy-5-phenylpentanoic, 6-hydroxy-6-phenylhexanoic, and 2-hydroxy-5-phenylpentanoic acids, carried out in this laboratory, indicated that these compounds are metabolized by rats to the extent of 30–100% of the administered dose. Therefore, these compounds were considered unsuitable for the proposed studies.

Determination of the Dissociation Constants, Apparent Partition Coefficients of the Compounds, and Their Binding to Whole Blood (Rat)—Values of pKa determined for the compounds used in

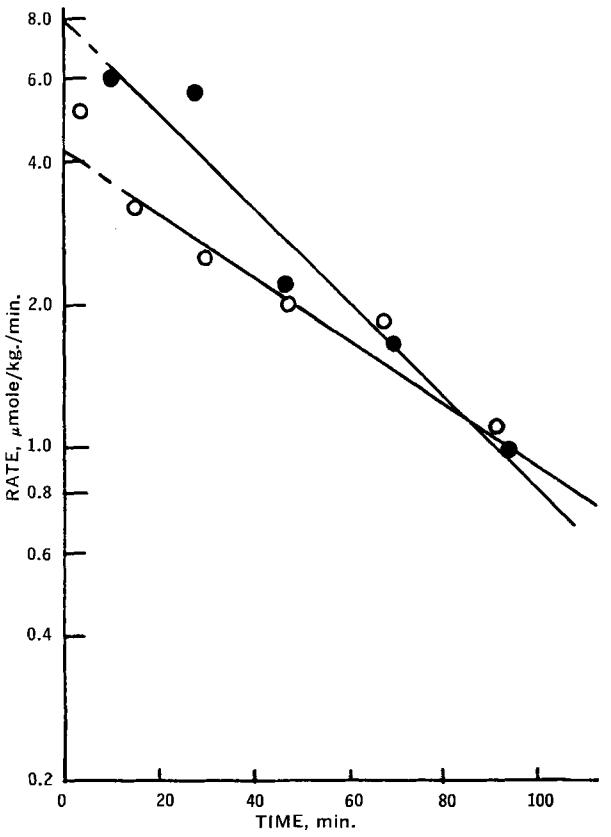

Figure 2—*Apparent first-order urinary excretion of* D-(−)-*mandelic acid following its i.v. administration of 367-μmole/kg. dose in the absence* (●) *and 346-μmole/kg. dose in the presence* (○) *of a 1.2 × 10⁴ μmole/kg. dose of* DL-*tropic acid to rats.*

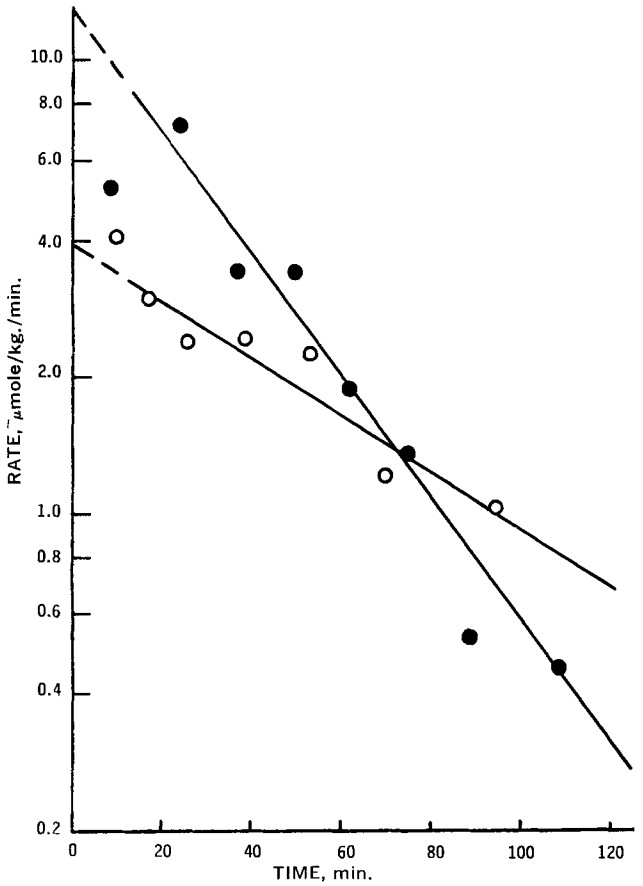

Figure 3—*Apparent first-order urinary excretion of* DL-*tropic acid following its i.v. administration of 410-μmole/kg. dose in the absence* (●) *and 344-μmole/kg. dose in the presence* (○) *of a 1.3 × 10⁴ μmole/kg. i.p. dose of* D-(−)-*mandelic acid to rats.*

the present studies are listed in Table II. These values are found to be in the range of 3.3 to 4.7. Since this range of pKa values is appreciably lower than the range of pH values (6.5–7.5) of the urine of the control rats used in all of the studies, it suggests that these compounds exist in the urine primarily in the ionized form and, therefore, are not likely to be significantly reabsorbed from the urine at the distal renal tubules. It is also evident from the results of the partition studies (Table II) that, except for the slight solubility of 4-hydroxy-4-phenylbutanoic acid, none of the compounds used in these studies is soluble in the lipidlike phase. Therefore, it may be assumed that none of these compounds is subject to significant renal tubular reabsorption from the urine at pH 6.5–7.5.

The results of the binding studies indicated that D-(−)-mandelic acid or any of its homologs used in the present studies is negligibly bound by the whole blood. This was deduced from the fact that, at equilibrium, the concentration of D-(−)-mandelic acid or its homolog in the solution outside of the dialysis bag containing the blood was the same as in the solution outside the dialysis bag of the control dialysis setup. The concentration of 1 mg. of D-(−)-mandelic acid or its homolog per dialysis setup used in the binding studies was based on the expected concentration of the acid in the volume of distribution of a rat weighing 200 g., following the intravenous administration of a 10-mg. dose of the acid. As suggested for humans (26), about 20% of the body weight was assumed to be the volume of distribution for rats. It was further assumed that the concentration of the compound in the blood and in the other possible volumes of distribution in rats is the same. The binding of D-(−)-mandelic acid by rat plasma in this concentration range has been studied and shown to be negligible (27).

Determination of the Biological Half-Life of the Compounds—It has been shown that, in humans, the metabolism and excretion of most drugs follow an apparent first-order process (28). Bray *et al.* (29) have demonstrated that the metabolism of benzaldehyde and toluene to benzoic acid and the urinary excretion of benzoic acid proceed by a pseudo-first-order process in rabbits. The urinary excretion of DL-tropic acid has also been shown to follow pseudo-first-order kinetics in rats (25). Although the urinary excretion of D-(−)-mandelic acid (14, 15) and L-(+)-mandelic acid (14) has been shown to occur by a pseudo-first-order process in humans, such studies have not been reported in rats for D-(−)-mandelic acid; D-(−)-4-hydroxy-4-phenylbutanoic acid; D-, L- or DL-phenyllactic acid; and D-(−)-benzyllactic acid. Therefore, it was necessary to determine if the excretion of D-(−)-mandelic acid and its homologs employed in the present studies follows pseudo-first-order kinetics. After establishing the proper procedures for rat urine collection and quantitative determination of these compounds excreted in the urine, the kinetics of urinary excretion of each compound were studied in rats. Preliminary data obtained in these studies indicated that the urinary excretion of these compounds occurred by a pseudo-first-order process.

Since the compounds used in the kinetic studies were those excreted unchanged in the urine of rats, the elimination of the compounds can be described by the following model:

$$A \xrightarrow{k} Ae$$

where A is the amount of intact compound in the body at any time, Ae is the amount of intact compound excreted in the urine at any time, and k is the apparent first-order rate constant of excretion of the compound. The urinary excretion data obtained for the compound can then be treated according to the following equation (15):

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

where $\Delta Ae/\Delta t$ is the rate of excretion of the compound at time t, and A_0 is the amount of the compound at zero time.

The log $\Delta Ae/\Delta t$ is plotted against t; from the slope of the straight line, obtained by the method of least squares, the biological half-

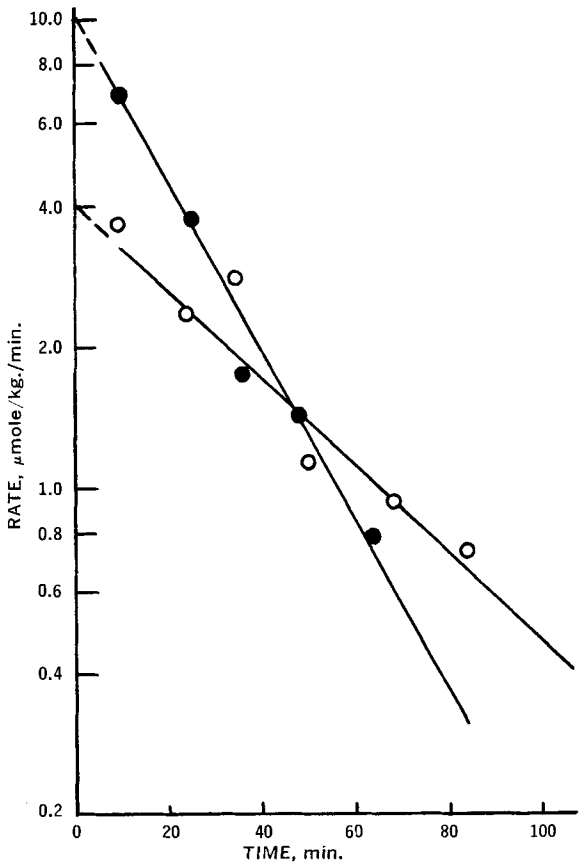

Figure 4—*Apparent first-order urinary excretion of* D-(−)-*4-hydroxy-4-phenylbutanoic acid following its i.v. administration of 277-μmole/kg. dose in the absence* (●) *and 277-μmole/kg. dose in the presence* (○) *of a 1.3 × 10⁴ μmole/kg. i.p. dose of* D-(−)-*mandelic acid to rats.*

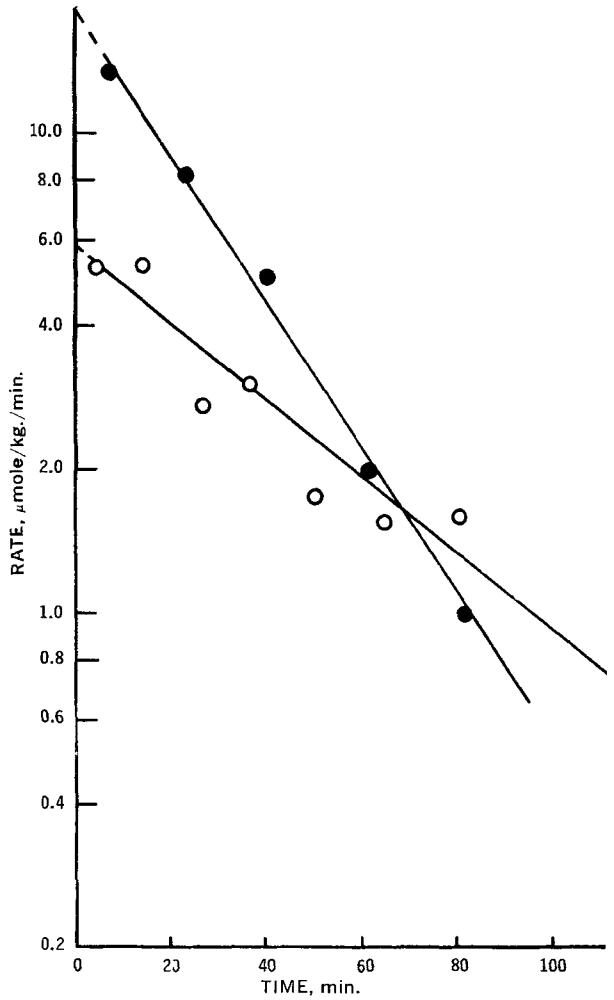

Figure 5—*Apparent first-order urinary excretion of* DL-*phenyllactic acid following its i.v. administration of 486-μmole/kg. dose in the absence* (●) *and 365-μmole/kg. dose in the presence* (○) *of a* 1.3×10^4 *μmole/kg. i.p. dose of* D-(−)-*mandelic acid to rats.*

life of the compound is calculated. The time, t, in such plots represents the midpoints of the urinary collection intervals.

One factor that should be considered in evaluating the excretion rate and biological half-life of compounds is the dose administered to the subject. This is important because the biological half-life of compounds involved in tubular secretion is expected to be longer at the dosage level that saturates the secretory process than at the dosage level that does not saturate this process. Therefore, for the determination of the biological half-life of a compound excreted by an apparent first-order process, its excretion should be studied following the administration of a dose that does not saturate the tubular secretory process. Consequently, the kinetics of urinary excretion of D-(−)-mandelic acid, DL-tropic acid and its optical isomers, D-(−)-4-hydroxy-4-phenylbutanoic acid, DL-phenyllactic acid and its optical isomers, and D-(−)-benzyllactic acid were studied following the intravenous administration of 150–700 μmole/kg. of each compound. The plots of log $\Delta Ae/\Delta t$ *versus* t for these studies were constructed, and from each straight-line plot the biological half-life of the corresponding compound was determined (Table III). The representative plots of log $\Delta Ae/\Delta t$ *versus* t obtained for each of these compounds are shown in Figs. 2–6.

It may be noted from Table III that the values of the biological half-life for DL-tropic acid and each of its optical isomers are similar to each other. Likewise, the values of the biological half-life obtained for DL-phenyllactic acid and each of its optical isomers are similar to each other. This indicated that the renal tubular transport of these compounds by the carrier mechanism is not stereospecific. Therefore, DL-tropic acid and DL-phenyllactic acid, instead of their individual optical isomers, were employed in the subsequent studies. The assignment of the absolute configuration of the compounds used in the study was based on their plain optical rotatory dispersion (ORD) curves as follows. Since the absolute configuration of (−)-mandelic acid is shown to be D-(−)-mandelic acid (30), the ORD curves for the homologs of mandelic acid were obtained over the wavelength range of 600–220 λ, and each curve was compared with that for D-(−)-mandelic acid. It was observed that, except for (−)-phenyllactic acid, the sign and shape of the ORD curves of the levorotatory homologs of mandelic acid were similar to that of D-(−)-mandelic acid. Thus, L-configuration was assigned to (−)-phenyllactic acid and D-configurations to the other levorotatory homologs of mandelic acid. This approach is in accordance with that described by Djerassi (31).

Among the compounds studied, the average biological half-life of D-(−)-mandelic acid is the longest (30 min.) and that of D-(−)-4-hydroxy-4-phenylbutanoic acid is the shortest (16 min.). The average half-life values for DL-phenyllactic acid and D-(−)-benzyllactic acid are not found to be significantly different from each other (20–21 min.). The average half-life for DL-tropic acid was found to be 23 min. (Table III). It is evident that the biological half-lives of the homologs of mandelic acid employed in these studies are significantly shorter than that of D-(−)-mandelic acid. Since the pKa values and the lipid solubility at the physiological pH determined for D-(−)-mandelic acid and its homologs are not significantly different, the differences observed in the values of their biological half-lives may be attributed to the increased hydrophobic interaction between the methylene group(s) of the mandelic acid homolog and a certain hydrophobic portion of the carrier for renal tubular secretion. Such hydrophobic interaction is likely to result in the increased affinity of the homolog for the carrier. In view of the fact that the initial excretion rate is composed of two terms, the initial glomerular filtration rate and the initial secretion rate, this interpretation of the biological half-life data would be acceptable

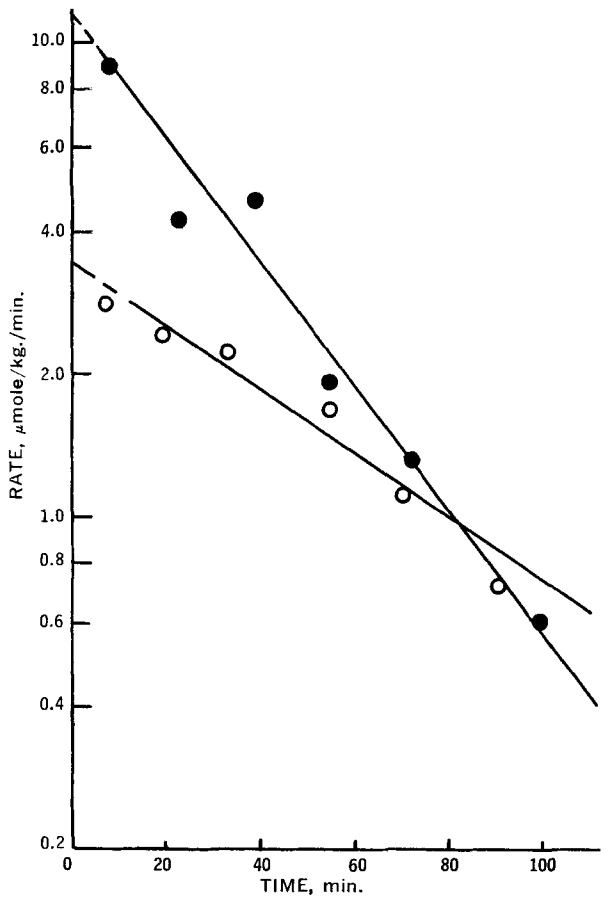

Figure 6—*Apparent first-order urinary excretion of* D-(−)-*benzyllactic acid following its i.v. administration of 346-μmole/kg. dose in the absence* (●) *and 298-μmole/kg. dose in the presence* (○) *of a* 1.3×10^4 *μmole/kg. dose of* D-(−)-*mandelic acid to rats.*

Table III—Summary of the Biological Half-Lives Observed in Rats for D-(−)-Mandelic Acid and Its Homologs[a] in the Absence and Presence of Inhibitor

Compound (Substrate) R = 		Biological Half-Life, min.			
			Inhibitor Present		
		Inhibitor Absent[b]	Equivalent[c] i.v. Dose	$10–15 \times 10^3$ μmole/kg. i.p. Dose	Inhibitor
D-(−)-Mandelic acid	R—CH(OH)—COOH	30 ± 4[d]	30 ± 4[d,e]	46 ± 8	DL-Tropic acid
DL-Tropic acid D-(−)-Tropic acid L-(+)-Tropic acid	$CH_2(OH)$ R—CH—COOH	23 ± 4 24 (2)[f] 25 (4)	28 ± 5	50 ± 10	D-(−)-Mandelic acid
DL-Phenyllactic acid L-(−)-Phenyllactic acid D-(+)-Phenyllactic acid	R—CH_2—CH(OH)—COOH	20 ± 3 21 (2) 20 (2)	26 ± 4	42 ± 5	D-(−)-Mandelic acid
D-(−)-Benzyllactic acid	R—$(CH)_2$—CH(OH)—COOH	21 ± 3	18 ± 2	36 ± 4	D-(−)-Mandelic acid
D-(−)-Hydroxy-4-phenylbutanoic acid	R—CH(OH)—$(CH_2)_2$—COOH	16 ± 2	22 ± 5	26 ± 5	D-(−)-Mandelic acid

[a] I.v. dosage range 150–700 μmole/kg. [b] The biological half-lives of the homologs of D-(−)-mandelic acid are significantly ($p<0.01$) shorter than that of D-(−)-mandelic acid. The biological half-life of D-(−)-4-hydroxy-4-phenylbutanoic acid is significantly shorter than that of DL-tropic acid ($p < 0.01$), DL-phenyllactic acid, and D-(−)-benzyllactic acid ($p < 0.05$). The biological half-lives of DL-phenyllactic acid and D-(−)-benzyllactic acid are not significantly shorter than that of DL-tropic acid. [c] The dose of inhibitor equivalent to the i.v. dose of the substrate compound. [d] Standard deviation was determined from six to eight rat studies. [e] The biological half-life of D-(−)-mandelic acid varied from 30 ± 3 to 33 ± 3 min. in the presence of equivalent i.v. doses of the other homologs of D-(−)-mandelic acid as inhibitors. [f] The values of average biological half-life of the individual optical isomers of tropic acid and phenyllactic acid based on the number of studies indicated in parentheses.

if the initial glomerular filtration rates of D-(−)-mandelic acid and its homologs are shown to be similar at the equimolar dosage levels. The data in support of this interpretation are presented in the subsequent discussion as well as in the next paper of this series (17).

Determination of the Apparent Initial Rate of Excretion—As in the case of biological half-life, a comparison was considered desirable between the apparent initial urinary excretion rates observed for compounds employed in the studies in terms of μmole/kg./min. However, it was realized that, since it is difficult to obtain urine from rats at such short intervals as 1 min. following the intravenous administration of the compound, the determination of the apparent excretion rate of substrate in terms of μmole/kg./min. was not feasible. Therefore, it was decided to determine the apparent initial urinary excretion rate in the above terms from an extrapolation of the straight-line plot of log $\Delta Ae/\Delta t$ *versus* time to zero time. Actually the initial urinary excretion rate should be obtained by an extrapolation of the straight line to the time of 0.5 min., because this would represent the midpoint of the urine collection interval. But this value of the excretion rate was very close to that obtained by an extrapolation to zero time, and the value of the intercept determined upon extrapolation of the straight-line plot to zero time was designated as the apparent initial urinary excretion rate. The apparent initial excretion rates determined in this manner for these compounds are listed in Table IV. It is noted that, in this dosage range, the apparent initial excretion rate of D-(−)-mandelic acid is considerably lower than the apparent initial excretion rate of any of its homologs used in this study. However, the differences in the apparent initial excretion rates of the homologs of D-(−)-mandelic acid are not readily seen in this dosage range (300–400 μmole/kg.) of the compounds.

Mutual Inhibitory Urinary Excretion Studies—To determine if the compounds under study are secreted by the same renal tubular transport mechanism, mutual inhibitory urinary excretion studies were carried out for DL-tropic acid, D-(−)-4-hydroxy-4-phenylbutanoic acid, DL-phenyllactic acid, and D-(−)-benzyllactic acid in the presence of D-(−)-mandelic acid. An isotonic solution containing a dose of 330–500 μmole/kg. of an appropriate homolog of mandelic acid and an equivalent amount of D-(−)-mandelic acid was administered intravenously to the rat; from the apparent first-order urinary excretion data obtained in each study, the biological half-lives of each homolog of mandelic acid and that of D-(−)-mandelic acid were determined (Table III). It is observed

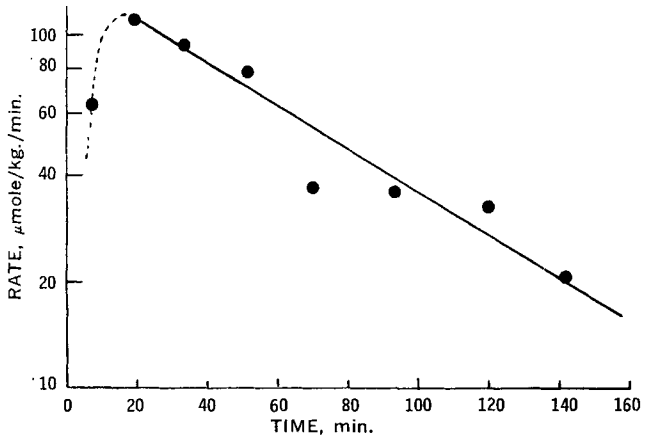

Figure 7—*Urinary excretion of* D-(−)-*mandelic acid observed following its i.p. administration of 1.0* × 10^4 *μmole/kg. dose to rats, indicating that the maximum absorption of the compound occurred in 20 min.*

Table IV—Comparison of Apparent Initial Urinary Excretion Rates Obtained in Rats for D-(−)-Mandelic Acid and Its Homologs[a] in the Absence and Presence of Inhibitor

Compound (Substrate)	Initial Excretion Rate, μmole/kg./min.		
	Inhibitor Absent	Inhibitor Present[b] (i.v. Equivalent[c] Dose per Rat)	Inhibitor Present[b] (i.p. 10–15 × 10^3 μmole/kg.)
D-(−)-Mandelic acid	5.0–8.5	6.1–7.9	3.8–5.8
DL-Tropic acid	11.8–16.4	6.9–9.0	3.5–4.3
DL-Phenyllactic acid	9.2–13.5	7.3–10.7	4.7–5.6
D-(−)-Benzyllactic acid	8.7–15.2	11.9–14.3	3.5–4.5
D-(−)-4-Hydroxy-4-phenylbutanoic acid	10.6–11.9	6.6–11.4	3.9–5.5

[a] I.v. dosage range of 300–400 μmole/kg. [b] DL-Tropic acid was employed as the inhibitor for D-(−)-mandelic acid, and D-(−)-mandelic acid was employed as the inhibitor for the remainder of the compounds. [c] Dose equivalent to that of the i.v. dose of the substrate compound.

from these data that, while the biological half-life of D-(−)-mandelic acid remained unchanged in the presence of each of its homologs, the biological half-life of each homolog, with the exception of D-(−)-benzyllactic acid, increased slightly in the presence of D-(−)-mandelic acid. Consequently, it was expected that there should be a corresponding slight decrease in the apparent initial rate of excretion of each homolog in the presence of D-(−)-mandelic acid. But because of the unavailability of data regarding the initial excretion rate of the compounds at identical doses (μmole/kg.), such a comparison was not possible. However, an attempt is made to compare the range of apparent initial excretion rates observed for the compounds over a dosage range of 300–400 μmole/kg. (Table IV). It can be seen from Table IV that, although there is a slight decrease in the apparent initial rate of excretion of DL-tropic acid and DL-phenyllactic acid, such a decrease in the rate is not observed for D-(−)-4-hydroxy-4-phenylbutanoic acid. Therefore, studies were carried out to determine the effect of higher doses of D-(−)-mandelic acid on the biological half-life and the apparent initial rate of excretion of each of its homologs following the administration of 300–400 μmole/kg. The dose of D-(−)-mandelic acid employed as an inhibitor in the studies was 1.3×10^4 μmole/kg., and it was administered intraperitoneally 15–20 min. prior to the intravenous administration of the substrate homolog. To determine the effect of a large dose of one of the homologs on the biological half-life of D-(−)-mandelic acid, similar studies were performed using DL-tropic acid as the inhibitor. The dose of DL-tropic acid used was 1.5×10^4 μmole/kg. and that of D-(−)-mandelic acid was 330 μmole/kg. The biological half-lives and apparent initial excretion rates determined for these compounds from the apparent first-order urinary excretion data are presented in Tables I and IV, respectively. The typical apparent first-order plots obtained for the compounds are shown in Figs. 2–6. The intraperitoneal administration of the high doses of DL-tropic acid and D-(−)-mandelic acid used as the inhibitor did not produce apparent toxic effects in rats. The rats behaved normally during the experiments as well as a week later when reused in the studies.

The pH of the urine of the rats following the administration of large doses of the inhibitor compounds was found to be in the range of 6.5 to 7.5, which was comparable to the urine pH of the control rats. The 15–20-min. interval between the administration of the inhibitor and the substrate was allowed to permit maximum absorption of the inhibitor, as shown by the studies on the urinary excretion of D-(−)-mandelic acid and DL-tropic acid after their intraperitoneal administration of $1.0–1.5 \times 10^4$ μmole/kg. (Fig. 7).

It is evident from the data presented in Tables I and IV that in the presence of a large dose of the inhibitor, there is a significant increase in the biological half-life and a corresponding decrease in the apparent initial excretion rate of each of the compounds employed in these studies. Therefore, it may be concluded that D-(−)-mandelic acid and its homologs, DL-tropic acid, D-(−)-4-hydroxy-4-phenylbutanoic acid, DL-phenyllactic acid, and D-(−)-benzyllactic acid, are involved in kidney tubular secretion and that they most probably share the same transport system for their secretion.

SUMMARY AND CONCLUSIONS

Studies were carried out in rats to gain some insight into the structural and chemical specificity that the "carrier" molecules of renal tubular transport show toward anionic drugs. D-(−)-Mandelic acid and certain of its homologs, DL-tropic acid, DL-phenyllactic acid, D-(−)-benzyllactic acid, and D-(−)-4-hydroxy-4-phenylbutanoic acid, were selected as the model compounds since they exhibit negligible metabolism and protein binding, low pKa values, and low lipid solubility at the physiological pH, and are primarily recovered in the urine in the unchanged form.

From the apparent first-order urinary excretion studies of these compounds, it was found that the biological half-life of each homolog is significantly shorter than that of D-(−)-mandelic acid in rats.

These compounds are shown to exert a mutual inhibitory effect on their renal tubular secretion in rats, indicating that they share the same carrier mechanism for their secretion.

The utilization of these compounds has demonstrated that the addition of methylene group(s) in the vicinity of the carboxyl group of the mandelic acid molecule increases its affinity for the carrier molecules of the renal transport system in rats.

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