Kinetics of Elimination of Optical Isomers of Mandelic Acid and Effect of Probenecid on Their Elimination Kinetics in Humans

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Abstract □ Kinetics of metabolism and urinary excretion of the optical isomers of mandelic acid have been studied in three human subjects, and the rate constants have been determined for a model consisting of parallel apparent first-order processes for excretion of intact mandelic acid and metabolism to benzoylformic acid. The kinetic studies revealed no significant difference in the rate of urinary excretion of L-(+)- and D-(−)-mandelic acid, but there was a difference in the rate of metabolism of these two isomers in the subjects. The rate constant for metabolism of L-(+)-mandelic acid was approximately twice that for the metabolism of D-(−)-mandelic acid. The inhibitory effect of probenecid on urinary excretion of the optical isomers of mandelic acid suggests that both isomers are involved in active renal tubular secretion.

Keyphrases □ Mandelic acid optical isomers—elimination kinetics □ Probenecid effect—mandelic acid optical isomers elimination □ Metabolism—mandelic acid optical isomers □ GLC—analysis

Although metabolism and urinary excretion of mandelic acid have been the subjects of considerable investigation in both humans and lower animals for more than 50 years, there has been no previous study of the kinetics of these processes. Schotten (1), Knoop (2), Neubauer and Fisher (3), and Quick (4) showed that mandelic acid fed to dogs was excreted unchanged in the urine. Similar observations were made in humans by Rosenheim (5). However, Wrede (6), Scholz (7), and Montenbruck (8) claimed that mandelic acid undergoes significant biotransformation both in man and dogs. Prior to 1938 the urinary excretion of only the racemic mandelic acid was studied, but Wrede (6), Scholz (7), and Montenbruck (8) also investigated the excretion of the individual optical isomers of mandelic acid. Montenbruck (9) extensively investigated the fate of individual optical isomers of mandelic acid in man. He found that 65% of the administered dose of L-(+)-mandelic acid was excreted in the urine as intact dextrorotatory mandelic acid, 14% as benzoylformic acid, and 7% as levorotatory phenylaminoacetic acid. From his studies with D-(−)-mandelic acid, Montenbruck observed that 73% of the administered dose was excreted as intact levorotatory mandelic acid, 7% as benzoylformic acid, and only traces as levorotatory phenylaminoacetic acid. Montenbruck also suggested that benzoylformic acid formed in the body by oxidation of either optical isomer of mandelic acid was oxidized to yield about 2% benzoic acid.

Gary and Smith (10) investigated the urinary excretion of racemic mandelic acid in decerebrate cats, and they reported that the initial urine samples showed a preponderance of levorotatory mandelic acid and that the later urine samples contained mainly the dextrorotatory mandelic acid. Similar differing excretion of optical isomers of mandelic acid was also observed in humans by Klingmüller and Brune (11), who reported that 74% of the administered dose of mandelic acid appeared in the urine in 24 hr., regardless whether the drug was administered orally or intravenously. Neish (12) reported that 13% of the administered dose of racemic mandelic acid was excreted in the urine as benzoylformic acid within 24 hr.

All these investigators used a polarimetric method to determine mandelic acid excreted in the urine. This method, however, was shown to exhibit an error as high as 15% (13). Other analytical methods reported for mandelic acid include gravimetric (13), iodometric (14), polarographic (15), and cerimetric (16). With the exception of the gravimetric method, these procedures involve conversion of mandelic acid to benzaldehyde and are not convenient for estimation of intact acid excreted in the urine. Williams (17) demonstrated that mandelic acid can be separated from urinary aromatic acids by gas chromatography on an 8% ethylene glycol adipate column.

The isomers of many optically active drugs are known to show differences in the extent of their metabolism and in the pharmacological responses they elicit upon administration to humans. These differences could be attributed to their different rates of absorption, metabolism, and urinary excretion. Since a significant fraction of an oral dose of mandelic acid is excreted in the urine as intact drug, this compound provides a convenient model for the investigation of possible differences in the rates of metabolism and urinary excretion. Also, many organic acids are actively secreted (18) by the renal

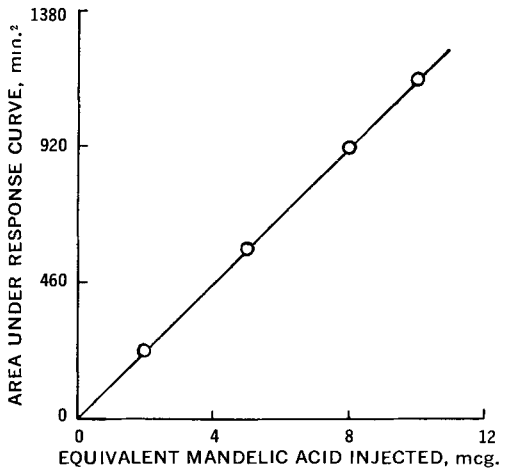


Figure 1—*Calibration curve for methyl ester of mandelic acid. Conditions: range, 10; attenuation, 64×; injection port temperature, 220°; column temperature, 180°; detector temperature, 250°; and helium flow, 80 ml./min.*

tubules; therefore, it is conceivable that this active transport might also show specificity with respect to the optical isomers of this model compound.

The specific objectives of the present study were to: (*a*) develop a gas chromatographic procedure that allowed the determination of both intact mandelic acid and its metabolites; (*b*) utilize the urinary excretion data to study the kinetics of, and determine the rate constants for, metabolism and urinary excretion of individual optical isomers of mandelic acid for the purpose of determining any differences in the metabolism and urinary excretion of the respective isomers in humans; and (*c*) determine the effect of probenecid on the excretion kinetics of the individual isomers of mandelic acid as a test for difference in active renal tubular transport for the two isomers.

EXPERIMENTAL

Materials—The following were used: L-(+)-mandelic acid,[1] recrystallized, m.p. 132–133°, $[\alpha]_D^{25}$ + 153°; D-(−)-mandelic acid,[1] recrystallized, m.p. 132–133°, $[\alpha]_D^{25}$ − 154°; benzoylformic acid,[1] m.p. 64°; benzoic acid USP,[1] m.p. 122°; and probenecid (500 mg).[2]

Apparatus—The gas chromatograph (F & M model 810) was equipped with a hydrogen-flame detector. The column used was a copper tube, 1.83 m. (6 ft.) long and 0.63 cm. (0.25 in.) outside diameter, packed with diatomaceous earth[3] coated with 8% ethylene glycol adipate. Helium was used as the carrier gas. A Beckman model G pH meter was used for all pH determinations.

Subjects and Test Procedures—Three, apparently healthy, male adult volunteers participated in this study. Each subject, after overnight fasting, ingested a given dose of an appropriate isomer of mandelic acid, dispensed in hard gelatin capsules, with about 250 ml. water. The subject was advised against eating anything for at least 2 hr. after ingesting the drug, but no dietary restrictions were imposed. Each subject collected a blank urine sample prior to ingestion of the drug. Following the ingestion of mandelic acid, urine was collected quantitatively at an interval of 1 hr. for 6 hr., at 2-hr. intervals up to 12 hr., and at longer intervals up to 48 hr. The urine samples were allowed to attain room temperature; then volume and pH were measured, and the samples were stored in the refrigerator until the time they were analyzed. No attempt was made to control the pH of urine during excretion studies. The pH of urine generally remained about 6 ± 0.5 throughout the study. Each subject ingested each of the isomers of mandelic acid in separate studies, generally after an interval of 1 week.

When the studies were carried out with probenecid, the subject ingested 1 g. of an appropriate isomer of mandelic acid after overnight fasting and collected urine quantitatively every hour for 5 or 6 hr. At the end of this period, immediately after collecting the urine sample, the subject ingested a hard gelatin capsule containing a powdered, 250 or 500 mg., probenecid tablet. The subject was advised to eat a light breakfast 2 hr. after ingestion of the dose of mandelic acid and was also advised against eating anything for at least 1 hr. after ingesting the dose of probenecid. Hourly urine samples were collected for another 5 or 6 hr. after ingestion of the probenecid, and subsequent urine samples were collected at longer intervals of time for 36–48 hr. The volume and pH of urine samples were recorded in the usual manner.

Analytical Method—Prior to analysis, urine samples were removed from the refrigerator and allowed to attain room temperature. An appropriate volume of each urine sample was pipeted into a suitable separator, 2.5 ml. of 5 *N* HCl was added to it, and mandelic acid and its metabolites were completely extracted with ether. The volume of the ether used for each of four extractions was about twice the volume of the aqueous phase. Each of the ether extracts was transferred to a 50-ml. beaker, and the ether was evaporated on a water bath at 55°. The residue was dissolved in 2 ml. methanolic sulfuric acid reagent (30 ml. concentrated sulfuric acid diluted to 200 ml. with anhydrous methanol) and transferred quantitatively to a 25-ml. glass-stoppered volumetric flask. The beaker was rinsed with two additional 2-ml. volumes of the reagent to transfer quantitatively the residue from the beaker to the flask. The flask was then stoppered, and the bulb of the flask was half-immersed in an oil bath at 75°, leaving the rest of the flask exposed to the atmosphere to serve as an air condenser for the methanol. After refluxing for 2 hr., the contents of the flask were cooled to room temperature, diluted with 5 ml. of distilled water, and transferred quantitatively to a 60-ml. separator with the aid of 20 ml. ether. After shaking and equilibration, the ether layer was transferred to a 50-ml. beaker and evaporated on a water bath at 55°. The aqueous alcoholic layer was further extracted three times with 20-ml. portions of ether, and the ether extracts were treated as described previously. The evaporation of ether was discontinued when approximately 0.3 ml. solution remained in the beaker. The residue was quantitatively transferred to a graduated test tube by rinsing the beaker with small portions of anhydrous methanol, and volume was adjusted to 5 ml. One microliter of this solution was injected onto the gas chromatographic column. The conditions employed for the gas chromatographic analysis were similar to those described elsewhere (19).

Blank urine samples of all participants in these excretion studies were treated in exactly the same manner as the other samples, and 1 μl. of the methanol extract was injected onto the column.

Fifty-milliliter solutions, each containing 10, 25, 40, or 50 mg. of mandelic acid, were prepared in distilled water; the solution was adjusted to about pH 2 with hydrochloric acid. These samples were extracted with ether, the acid esterified with the methanolic sulfuric acid reagent, and prepared for gas chromatographic analysis in the same manner as the urine samples. These samples were prepared to construct a calibration curve for mandelic acid (Fig. 1).

RESULTS AND DISCUSSION

Gas Chromatographic Method for Quantitative Determination of Mandelic Acid and Its Metabolites—To render them suitable for gas chromatographic analysis, carboxylic acids are routinely converted to their methyl esters. Various agents reported for methylating carboxylic acids include diazomethane (17, 19, 20), BF_3-methanol (21–23), and dry methanolic HCl (24). Prior to the use of diazomethane as an esterifying agent of mandelic acid and its metabolites for their gas chromatographic analysis by Kamienny *et al.* (19) and Randinitis *et al.* (20), the method described here was used to esterify the acids. Considering the simplicity of preparation and use of dry methanolic HCl, this method was employed to esterify mandelic acid, but the low yield of the ester under the conditions employed necessitated development of another suitable reagent, namely, dry methanolic sulfuric acid. As evidenced in Fig. 1, 2 hr. of refluxing mandelic acid with this reagent offered a reproducible yield of the ester.

The gas chromatograms obtained for blank urine samples of all the participants of these excretion studies revealed complete absence of a peak at that retention time (12.2 min.) where the peak for methyl mandelate appears. Small peaks appeared at retention times between 4 and 6 min. This situation afforded an effective means of estimating benzoylformic acid, the principal metabolite of mandelic acid, which was previously identified by forming a 2,4-dinitrophenylhydrazone derivative in the urine samples voided after ingesting mandelic acid (12). Benzoylformic acid was prepared for gas chromatographic analysis in the same manner as mandelic acid, and a standard calibration curve was constructed. The retention observed for methyl benzoylformate was 8.2 min.

The blank urine sample treated with methanolic sulfuric acid rarely showed a peak at a retention time of 2.2 min., but the urine samples voided after ingesting mandelic acid and treated with the reagent often showed a peak at this retention time. Since methyl benzoate[4] exhibits a peak at this retention time, such a peak in a chromatogram was attributed to benzoic acid, which appears in the urine as the ultimate oxidation product of mandelic acid. A standard curve was also constructed for methyl benzoate.

Mandelic acid and its metabolites are thus extracted together from a urine sample and are esterified in a single esterification step with the methanolic H_2SO_4 reagent. Finally, when the ester extract

[1] Aldrich Chemical Co., Inc., Milwaukee, WI 53210
[2] Benemid tablets, Merck Sharpe and Dohme.
[3] Diatoport S, 80–100 mesh.
[4] Eastman Organic Chemicals, Rochester, N. Y.

Table I—Summary of Urinary Excretion Recovery Data Obtained in Human Subjects after Oral Administration of Each Optical Isomer of Mandelic Acid in the Absence and in the Presence of Orally Administered Probenecid

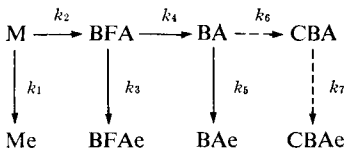
Subject	Oral Dose, mg.		Equivalent Mandelic Acid Excreted as—				Total Mandelic Acid Excreted, mg.
	Mandelic Acid	Probenecid	Intact Mandelic Acid, mg.	Benzoylformic Acid, mg.	Benzoic Acid, mg.	Percent Metabolized[a]	
			D-(−)-Mandelic Acid Alone				
A	1000	—	922	79	9	8.7	1010
B	990	—	878	65	16	8.4	959
C[b]	995	—	721	85	21	12.9	827
			L-(+)-Mandelic Acid Alone				
A	1000	—	801	208	—	20.6	1009
B	1000	—	848	147	23	16.7	1018
C[b]	1005	—	756	159	22	19.6	937
			D-(−)-Mandelic Acid + Probenecid[c]				
B	1015	250	978	66	—	6.3	1044
B	1420	500	1270	123	4	9.0	1397
			L-(+)-Mandelic Acid + Probenecid[c]				
B	1000	250	842	174	16	18.4	1032
B	972	250	701	174	5	20.2	880
B	1545	500	1270	304	27	20.7	1601
C[b]	1000	250	712	180	11	21.0	903

[a] The percentage is based on the total amount of mandelic acid recovered in the urine. [b] The subject inadvertently drank milk along with mandelic acid, which may account for incomplete recovery of mandelic acid. [c] Probenecid was administered 4.5 or 6 hr. after the ingestion of mandelic acid (Fig. 2).

is injected onto the polar column, the esters are separated on the column due to the difference in their polarity. Thus, a single injection of the sample makes the quantitative determination of intact mandelic acid and its metabolites possible, as previously shown (19). The area under the response curve for each of the respective acids was directly proportional to its concentration. The area under the response curve was calculated by the trapezoidal rule (25).

To determine accurately the amount of mandelic acid and its metabolites present in the urine samples, calibration curves of concentration *versus* area under the response curve for the respective acids were constructed each time the urine samples were analyzed. This analytical procedure produced results accurate to ±5%.

Model for Excretion Kinetics of Mandelic Acid and Determination of Rate Constants—The recovery of the administered dose of each optical isomer of mandelic acid was complete in the majority of the studies (Table I). Preliminary data obtained for excretion of intact mandelic acid following the oral administration of the respective isomer indicated that the elimination of the acid proceeded by an apparent first-order process. From the information thus obtained about the fate of mandelic acid in the human subjects, Scheme I was formulated to describe a kinetic scheme for metabolism of mandelic acid and its metabolites after absorption of drug and attainment of apparent equilibrium between the drug in blood and the drug in other fluids of distribution. In this scheme, M, BFA,



Scheme I

BA, and CBA are the respective amounts of mandelic acid, benzoylformic acid, benzoic acid, and conjugates of benzoic acid in the body at any time; Me, BFAe, BAe, and CBAe are the amounts of the respective compounds excreted in the urine at any time; and k_i (i = 1...7) represents apparent first-order rate constants for the respective processes indicated in the model. From 0 to 2% of the administered dose of D-(−)-mandelic acid and from 1 to 2% of the administered dose of L-(+)-mandelic acid were recovered in the urine as benzoic acid. Although glycine or glucuronic acid conjugates of benzoic acid are likely to be formed in the body (26), the extent of conjugation is considered negligible, since the amount of benzoic acid formed in the body is very small. Therefore, the conjugation step in the model is indicated by a broken line.

One primary purpose of the present studies was to determine the rate constants for metabolism and urinary excretion of the optical isomers of mandelic acid from the urinary excretion data. Therefore, the following differential equations were considered:

$$d\mathrm{Me}/dt = k_1\mathrm{M} = k_1\mathrm{M}_T e^{-k(t-\mathrm{T})} \quad \text{(Eq. 1)}$$

$$\log \Delta\mathrm{Me}/\Delta t = \log k_1\mathrm{M}_T - k(t - T)/2.303 \quad \text{(Eq. 2)}$$

where $\Delta\mathrm{Me}/\Delta t$ is the rate of urinary excretion of intact mandelic acid (mg./hr.); k, which is equal to $k_1 + k_2$, is the overall rate constant for disappearance of the drug from the body [referred to in recent literature as the disposition-rate constant (26)]; k_1 is the fraction of k attributable to processes leading to urinary excretion of intact drug; k_2 is the fraction of k attributable to processes leading to conversion of mandelic acid to the primary metabolite, benzoylformic acid; M_T is the amount of intact mandelic acid in the body at time T after oral administration of the drug; and T is the time period (hours) during which apparent complete absorption of drug occurred from the gastrointestinal tract and at the time, T, excretion of the drug became apparent first-order. The log $\Delta\mathrm{Me}/\Delta t$ was plotted against t and the rate constant, k, determined from the resulting straight line (least squares) obtained for the data after T hr. The T was generally less than 2 hr. in Subjects A and B, but it was 3–4 hr. in Subject C, who inadvertently drank milk along with the drug (Fig. 2). The time t in Figs. 2 and 3 represent the midpoints of the urinary collection intervals. The values of k_1 and k_2 were determined as follows:

$$k_1 = k \cdot f \quad \text{(Eq. 3)}$$

$$k_2 = k - k_1 \quad \text{(Eq. 4)}$$

$$f = \frac{\mathrm{Me}_\infty}{\mathrm{Me}_\infty + \mathrm{BFAe}_\infty + \mathrm{BAe}_\infty} \quad \text{(Eq. 5)}$$

where Me_∞, BFAe_∞, and BAe_∞ are the equivalent amounts of mandelic acid excreted in the urine as intact mandelic acid, benzoylformic acid, and benzoic acid during 48 hr., the experimental infinite time.

Comparison of Rate Constants for Metabolism and Urinary Excretion of Optical Isomers of Mandelic Acid—Comparison of data for the isomers of mandelic acid, as illustrated in Table II, indicates no significant differences in values of k and k_1. However, the value of k_2 in all subjects is found to be greater for L-(+)-mandelic acid than for D-(−)-mandelic acid. Table I also indicates that in all subjects the recovery of the metabolites for L-(+)-mandelic acid was about twice the recovery of metabolites observed for D-(−)-mandelic acid. Clearly, k_2 is a hybrid rate constant, involving processes of distribution as well as a specific rate of conversion of intact drug to benzoylformic acid at the metabolic site. However,

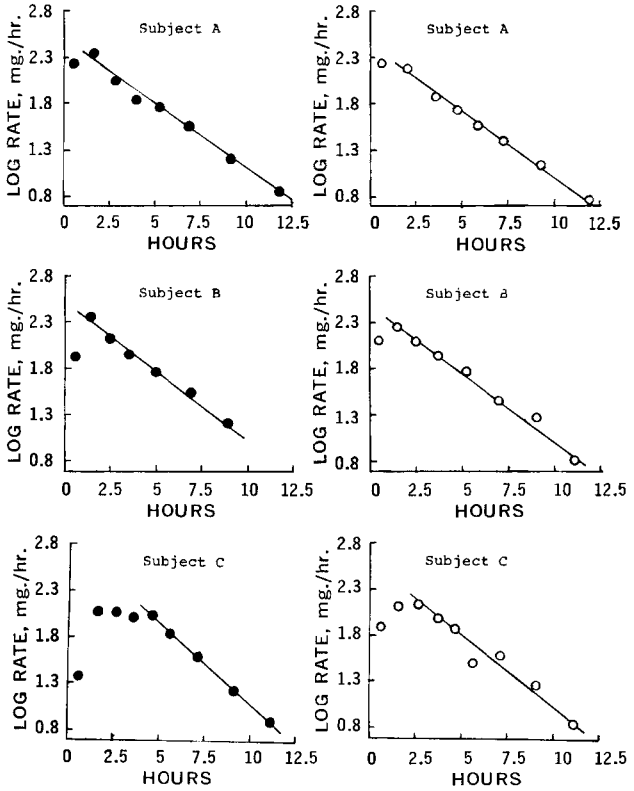


Figure 2—*Apparent first-order urinary excretion of* D-(−)-*mandelic acid* (●) *and* L-(+)-*mandelic acid* (○) *in Subjects A, B, and C. The prolonged periods for absorption of mandelic acids reflected in the plots of Subject C are probably caused by milk inadvertently drunk by the subject along with mandelic acid.*

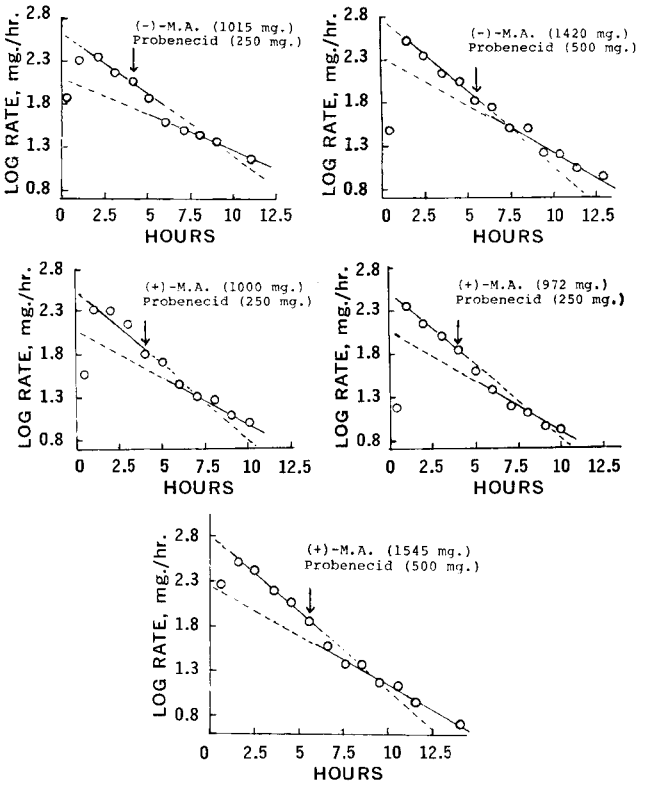


Figure 3—*Effect of probenecid on the urinary excretion of the optical isomers of mandelic acid (M.A.) in Subject B. The arrow indicates the time at which probenecid was ingested by the subject. The numbers in parentheses represent the doses of the corresponding compounds. The isomer of mandelic acid was ingested at zero time.*

since L-(+)- and D-(−)-mandelic acid have similar physical properties, if it is assumed that distribution within the body is accomplished primarily by processes other than active transport, it is probable that differences in k_2 for the two isomers do, in fact, represent differences in the rate at which intact drug is transformed at the metabolic site. It may then be concluded that, while the oxidative enzyme(s) show apparent differential affinity for the optical isomers of mandelic acid, the tubular "carrier" system does not show similar differential affinity for renal tubular secretion of these isomers in humans. The evidence indicating that both isomers of mandelic acid are involved in renal tubular secretion is presented in a later section.

Comparison of Quantitative Aspects of the Fate of Mandelic Acid Observed in Present Studies with Those Reported by Others—After the oral ingestion of L-(+)-mandelic acid, since the urine, following its exhaustive extraction with ether, exhibited levorotatory activity upon polarimetric examination, Montenbruck (9) assumed that the levorotation was caused by 7% (−)-phenylaminoacetic acid formed from mandelic acid in the human subject and eventually excreted in the urine. Furthermore, he reported that only traces of phenylaminoacetic acid were formed from D-(−)-mandelic acid, since he did not encounter similar observation in the urine collected following the oral ingestion of mandelic acid. In these studies the equivalent amount of either isomer of mandelic acid recovered from the urine as intact mandelic acid and benzoylformic acid was about 80% of the administered dose. However, the data presented in Table I indicate that the urinary recovery of either isomer of mandelic acid is practically complete (97–103% of the ingested dose) in most studies in Subjects A and B. Similar observation was also made by Kamienny *et al.* (19) in their studies with D-(−)-mandelic acid. Therefore, if (−)-phenylaminoacetic acid was formed from L-(+)-mandelic acid in the body, as suggested by Montenbruck (9), it was assumed to be negligible and no attempt was made to identify it.

Although the total urinary recovery of the administered dose of each mandelic acid isomer reported by Montenbruck (9) was less than that observed in the present study, a fairly good agreement is noted in these studies between the fractions of the dose of mandelic acid obtained as its metabolites (benzoylformic acid and benzoic acid). The extent of metabolism is indeed found to be greater for L-(+)-mandelic acid than for D-(−)-mandelic acid, and the urinary recovery of the former in the intact form is less than that of the latter. As shown by Montenbruck (9), not more than 2.3% of the administered dose of L-(+)-mandelic acid was obtained as benzoic acid in the present studies.

In their studies, Kamienny *et al.* (19) noted that the extent of metabolism of the administered dose of D-(−)-mandelic acid to benzoylformic acid was 10–15% in two of the three human subjects and 14–22% in the third subject. Subject B, who participated in those studies, was the same Subject B who participated in the present studies, and yet the subject was found to metabolize 6–8% of the administered dose of D-(−)-mandelic acid to benzoylformic acid in the present study (Table I) in contrast to 11–13% in the Kamienny *et al.* studies (19). The overall elimination rate constants observed for D-(−)-mandelic acid in Subject B prior to the administration of probenecid in the inhibitory studies were $33-36 \times 10^{-2}$ hr.$^{-1}$ (Table III), while in the Kamienny *et al.* studies (19) the overall elimination rate constants observed for D-(−)-mandelic acid in Subject B prior to the administration of sulfonamides were 44–46 $\times 10^{-2}$ hr.$^{-1}$. Furthermore, the two studies with D-(−)-mandelic acid (without probenecid and with 250 mg. probenecid) in Subject B were carried out 5 years ago (Table I); the studies with the same compound in the same Subject B were carried out by Kamienny *et al.* (19) 2 years ago, and the study with the same compound (with 500 mg. probenecid) in the same Subject B was carried out recently (Table I).

In retrospect, it is correctly recollected that, while Subject B was generally resting during the first 10–12 hr. of urine collection for the studies reported in Table I, he was ambulatory during the similar urine-collection period reported in the Kamienny *et al.* studies (19). Recently, Schmidt and Roholt (27) demonstrated that the initial plasma levels of benzylpenicillin following the intramuscular administration to the human subjects were higher when they were ambulatory than when they were resting in bed. Subjecting these data reported by Schmidt and Roholt to pharmacokinetic analysis, Levy (28) observed that, although the increase in the average rate

Table II—Apparent First-Order Rate Constants for Overall Elimination (k), for Processes Leading to Urinary Excretion (k_1), and for Processes Leading to Metabolism (k_2) of Optical Isomers of Mandelic Acid in Humans

Subject	k, hr.$^{-1}$ ($\times 10^2$)	k_1, hr.$^{-1}$ ($\times 10^2$)	k_2, hr.$^{-1}$ ($\times 10^2$)
	D-(−)-Mandelic Acid		
A	31.78	29.02	2.76
B	31.68	29.02	2.66
B[a]	33.39	31.29	2.10
B[a]	38.46	34.98	3.48
C	40.16	34.98	5.18
	L-(+)-Mandelic Acid		
A	32.31	25.66	6.65
B	33.57	27.97	5.60
B[a]	39.84	24.87	7.33
B[a]	38.13	30.43	7.70
B[a]	37.53	29.76	7.77
C	37.24	29.94	7.30
C[a]	37.90	29.94	7.96

[a] The rate constants presented for these subjects were determined from the urinary excretion data obtained prior to the administration of probenecid (Fig. 3). The reason for utilizing these data is explained in the text.

constant for overall elimination of the drug was only 1.1-fold, such an increase in the average rate constant for metabolism of the drug was 2-fold in the subjects when they were ambulatory. Therefore, the greater extent of the metabolism of D-(−)-mandelic acid noted by Kamienny *et al.* (19) for Subject B and others possibly was due to their ambulatory state during the study. Subject C (Tables I and II) was generally ambulatory during the studies, and this probably was a significant factor for the considerably higher rate of metabolism in Subject C than in Subjects A and B, especially in the study of D-(−)-mandelic acid. Further studies will be carried out for L-(+)-mandelic acid to determine the effect of an ambulatory state of the subjects during such studies.

Effect of Probenecid on Urinary Excretion of Optical Isomers of Mandelic Acid—Since probenecid is known to inhibit the renal tubular secretion of a large number of organic anions (18), studies were carried out to determine if probenecid is able to decrease the rate of urinary excretion of the optical isomers of mandelic acid. It was thought that such studies would reveal whether or not these compounds are involved in renal tubular secretion. In the event the optical isomers of mandelic acid are involved in renal tubular secretion, and their tubular secretion is suppressed by probenecid, it was further thought that the extent of inhibitory effect of probenecid on the urinary excretion of the compounds might indicate if the renal tubular transport carrier system shows differing affinity for the compounds.

As shown in Fig. 3 and Table III, the inhibitory effect of probenecid on the urinary excretion of each isomer of mandelic acid becomes evident. The rate constant (k) for overall elimination of an optical isomer in the absence of probenecid was calculated from the slope of the straight line (least squares) for the data obtained after the postabsorptive and postequilibrative period, but prior to the administration of probenecid. The modified rate constant (kp) for overall elimination of the optical isomer in the presence of probenecid was calculated from the slope of the straight line (least squares) of its excretion data obtained following the oral administration of probenecid. However, since the rate of excretion of the isomer at the 1st hr. after the oral administration of probenecid (Fig. 2) is unlikely to represent the excretion rate of the isomer in the period of postabsorption and postequilibration of probenecid in the body, it is excluded when constructing the least-squares straight line for the subsequent urinary excretion data to calculate the modified rate constant kp. The values of R obtained from the ratio k/kp are listed in Table III. The values of R are used as the index of the inhibitory effect of probenecid on the urinary excretion of the optical isomers of mandelic acid for the following reason.

It is noted from the summary of urinary excretion recovery data (Table I) that the recovery of the administered dose of the optical isomers of mandelic acid in the studies involving probenecid was generally complete and that the extent of metabolism of the isomers of mandelic acid in these studies was comparable to that observed

Table III—Rate Constants for Overall Elimination of Optical Isomers of Mandelic Acid Determined in Humans before (k) and after (kp) Oral Administration of Probenecid, and the Index of Inhibitory Effect ($R = k/kp$) of Probenecid on the Urinary Excretion of Optical Isomers of Mandelic Acid

Subject	Mandelic Acid Dose, mg.	Probenecid Dose, mg.	k, hr.$^{-1}$ ($\times 10^2$)	kp, hr.$^{-1}$ ($\times 10^2$)	R
		D-(−)-Mandelic Acid			
B	1015	250	33.39	20.22	1.65
B	1420	500	38.46	25.27	1.52
		L-(+)-Mandelic Acid			
B	1000	250	39.84	24.87	1.60
B	972	250	38.13	26.48	1.44
B	1545	500	37.53	25.81	1.45
C	1000	250	37.90	27.42	1.38

in absence of probenecid. The extent of metabolism of the isomers of mandelic acid appears unaffected in the presence of probenecid, since probenecid was administered to the subject 4.5 or 6 hr. after the administration of mandelic acid, during which period 80–85% of the administered dose of mandelic acid was eliminated. Therefore, even if the extent of metabolism of mandelic acid had probably increased in the presence of probenecid, the slight increase in the amount of metabolite due to the remaining dose (15–20% of the administered dose) of mandelic acid in the body is unlikely to become apparent when the total amount of metabolite due to the entire dose of mandelic acid is considered. Differences in the overall elimination rate constants (k and kp) observed in the inhibitory studies appear to be primarily attributable to the differences in the urinary excretion rate.

It is further concluded that the decrease in the rate of urinary excretion of the isomers of mandelic acid in the presence of probenecid is due to the decrease in the rate of secretion of the compounds caused by probenecid. The inhibitory studies described here were carried out primarily in Subject B; only one study was carried out in Subject C. Judging from the values of kp or R (Table III) determined for each isomer of mandelic acid, it does not appear that probenecid decreases the excretion rate of one isomer to a significantly greater extent than that of the other isomer at the dosage levels of the substrate compounds (mandelic acid isomers) and the inhibitor (probenecid) employed in the present study.

As already pointed out, the extent of metabolism of the optical isomers of mandelic acid remained apparently unaffected in the studies involving probenecid (Table I). Furthermore, the rate constants (k) determined for overall elimination of each optical isomer of mandelic acid in the studies involving probenecid (Table III) are found to be similar to those obtained for the isomers in the studies that did not involve probenecid (Table II). Therefore, the urinary excretion data obtained for each optical isomer of mandelic acid in the inhibitory studies (prior to the administration of probenecid) are utilized to determine the apparent rate constants, k_1 and k_2, of the respective isomers. The values of the rate constants thus determined are included in Table II, because these results may be considered as the results of the additional studies performed by the subjects in support of the results obtained in the studies involving only the respective isomer of mandelic acid.

In conclusion, it appears that, in humans, the "carrier" system responsible for renal tubular secretion of mandelic acid shows no apparent stereospecificity for the optical isomers of mandelic acid, as does the oxidative enzyme system for their metabolism.

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Effect of Vehicles on Metabolism of Serotonin and Imipramine

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Abstract □ Effects of different vehicles on excretion and metabolism of serotonin and imipramine were studied. Rats receiving serotonin-^{14}C in dimethyl sulfoxide or propylene glycol excreted radioactivity in the urine slower and in lesser amounts than when water was the vehicle. However, the urinary excretion of radioactivity by rats administered a single dose of imipramine-^{14}C was the same for the three vehicles over a period of 72 hr. Dimethyl sulfoxide and propylene glycol caused not only an increase in the amount of urinary 5-hydroxytryptophol and *N*-acetylserotonin, but also an increase in the conjugated form of these two metabolites as well as that of 5-hydroxyindoleacetic acid. These two nonaqueous vehicles caused a diminished demethylation of imipramine when compared to the water vehicle.

Keyphrases □ Serotonin-^{14}C metabolism—vehicle effect □ Imipramine-^{14}C metabolism—vehicle effect □ Metabolism—serotonin-^{14}C, imipramine-^{14}C □ Paper chromatography—autoradiographic analysis □ Scintillometry—analysis

An aqueous solvent is the most common vehicle for the administration of drugs for pharmacological studies in animals. However, other solvents are also frequently used, such as propylene glycol and dimethyl sulfoxide (DMSO). DMSO is a powerful solvent with a remarkable ability to alter membrane permeability, and its function as a carrier for many substances has been demonstrated by a number of investigators.

Recently, effects of DMSO on the uptake of ^{14}C-pemoline (1, 2), ^{14}C-urea, and ^{14}C-sucrose (3) by the rat brain have been reported. This study is to determine the difference in effects of water and these two organic solvents as vehicles on the metabolism of two ^{14}C-labeled centrally acting agents, serotonin and imipramine.

MATERIALS AND METHODS

Labeled Compounds—Serotonin-2-^{14}C creatinine sulfate[1] and imipramine-^{14}C hydrochloride[2] were used.

Animals and Doses—Male Sprague-Dawley rats, weighing between 180 and 220 g., were used. The compounds were dissolved separately in water, DMSO, and propylene glycol and were administered intraperitoneally to groups of three animals in a dose of 20 mg./kg. (0.5 ml./rat; 1 μc./rat).

Collection of Urine and Fecal Samples—After the administration of the compound, animals were kept in metabolism cages. Urine samples were collected at intervals of 2, 3, 4, 8, 12, and 24 hr. and then at every 24-hr. interval up to 6 days. In some cases the collection was extended beyond 6 days, as indicated in the results. Toluene was added to the collection tube to prevent any bacterial growth. Fecal samples were collected every 24 hr. for several days. All samples were immediately frozen until assayed.

Determination of Radioactivity—Radioactivities were measured with a Nuclear Chicago liquid scintillation spectrometer, model 725. The scintillation fluid was composed of 4 g. of PPO, 50 mg. of POPOP, and 70 g. of naphthalene/l. of toluene. All determinations were performed in duplicate and were corrected to 100% efficiency by the channels ratio method (4) and for recovery of radioactivity.

The urine sample (0.1 ml.) was mixed with 3 ml. of methanol in a counting vial, and the radioactivity was then measured with 15 ml. of the scintillation fluid..

For measuring the radioactivity in feces, 0.1 ml. of 20% water homogenate in a counting vial was treated with 1.0 ml. of 10% hydrogen peroxide in methanol. The mixture was heated at 45° to al-

[1] New England Nuclear Corp.
[2] Nuclear Chicago Corp.