Pharmacokinetics of Phosphonomycin in Man I: Intravenous Administration

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Abstract □ The disposition of phosphonomycin following intravenous administration is adequately described by a two-compartment open model. Pharmacokinetic parameters derived from serum concentration data correctly predict the urinary excretion profile for the same individual. Lack of self-consistency between serum and urine profiles at any time is in every case accompanied by apparent deviations in renal clearance rate. The adequacy of the model is further illustrated by the good agreement between serum and urinary excretion profiles predicted with pharmacokinetic parameters derived for individual subjects and those actually observed on repeated administration to the same subjects.

Keyphrases □ Phosphonomycin—pharmacokinetics □ Pharmacokinetics—phosphonomycin, intravenous administration □ Renal, serum clearance, human—phosphonomycin □ Distribution-rate constants, volumes, human—phosphonomycin

Phosphonomycin[1], (−)(1R,2S)-1,2-epoxypropylphosphonic acid, is a newly discovered antibiotic produced by strains of *Streptomyces*. Its isolation, characterization, synthesis, and biological properties have been the subjects of recent publications (1–7). Early pharmacologic observations following the oral and intravenous administration in man also have been reported (8–10).

The present communication is concerned only with the pharmacokinetic analysis of serum concentration and urinary excretion data from single and repeated intravenous administrations in apparently healthy, male volunteers. In particular, evidence is developed in support of the two-compartment open model in representing data for individual subjects who received single doses of 250 or 500 mg. of phosphonomycin as the disodium salt. Pharmacokinetic parameters derived for a given individual are shown to give reasonable predictions for the serum and urinary excretion profiles on the same individual on repetitive dosing. Apparent departures from the model, manifest as inconsistencies between serum and urine data, are in every case accompanied by deviations in measurements of serum renal clearance in the same direction. Throughout this discussion, emphasis is placed on self-consistency of the proposed model for individual subjects. Hopefully, the analysis will also help to demonstrate some aspects of pharmacokinetic modeling which heretofore have only been asserted.

EXPERIMENTAL

Pharmacokinetic analysis was performed on results of exploratory studies originally designed to determine the fate of phosphonomycin in man. The experimental design was not originally intended to include pharmacokinetic modeling, but the available data appeared to be sufficient to permit analysis. The data to be discussed were obtained in four separate studies involving apparently healthy, male volunteers. Some subjects participated in more than one study; in these instances, the same numeral identifications are retained throughout so as to facilitate comparison. In studies involving single intravenous injections, the dose of phosphonomycin as the lyophilized powder of the disodium salt was dissolved in 30 ml. of 5% dextrose and was injected into an antecubital vein in 10 min. or less. The procedure for the renal clearance study was already reported (8). Serum and urine specimens were bioassayed by a conventional cup-plate method, employing *Proteus vulgaris* (ATCC 21100) as the test organism.

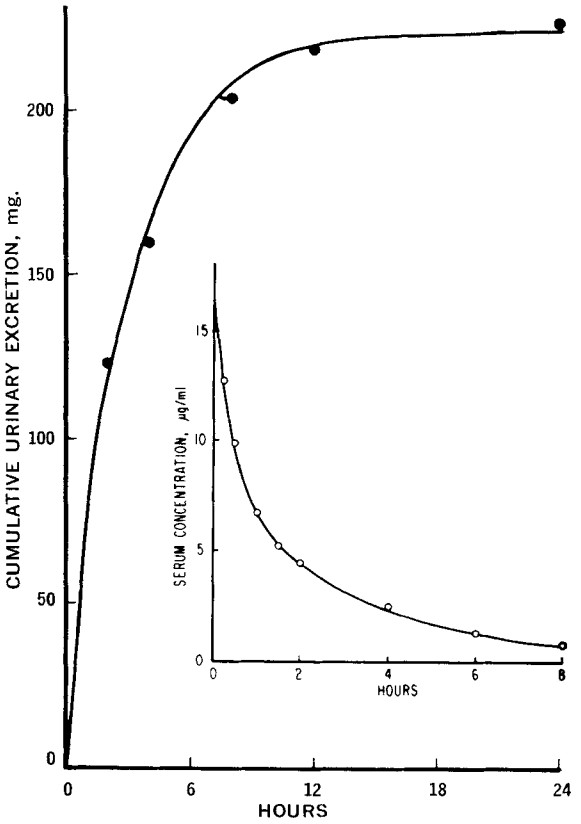


Figure 1—*Typical serum and urinary excretion profiles. Key: Observed serum* (○) *and urine* (●)*; predicted* (——). *Subject 7.*

Study 1—Eight subjects received 250 mg. of phosphonomycin as single injections. Blood samples were taken at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, and 8 hrs. Fractional urine samples were collected at intervals of 0–2, 2–4, 4–8, 8–12, ad 12–24 hr.

Study 2—Six subjects received 500 mg. of phosphonomycin as single injections. Blood samples were taken at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 hr. Urine samples were collected fractionally at intervals of 0–2, 2–4, 4–8, 8–12, 12–24, and 24–36 hr.

Study 3—Six subjects received 500-mg. doses of phosphonomycin repetitively at 6-hr. intervals as single injections. A total of eight doses was given. Three of the subjects (6, 10, and 13) had participated in either Study 1 or 2. The other three (15, 16, and 17) had participated in another study (not reported here) in which two 500-mg. doses were given 4 hr. apart as single injections; hence, their pharmacokinetic parameters were also known. Blood samples were taken at 0, 0.25, 1, 2, 6, 6.25, 12, 12.25, 18, 18.25, 24, 24.25, 30, 30.25, 36, 36.25, 37, 38, 40, 42, 42.25, and 48 hr. Fractional urine collections were made at 6-hr. intervals for a total of 72 hr.

[1] Phosphonomycin has recently been redesignated as fosfomycin by the United States Adopted Names Council.

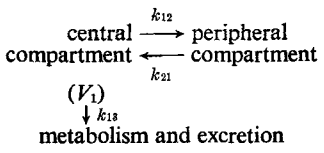
Table I—Estimated Rate Constants and Volumes of Distribution for Phosphonomycin in Man

Subject	Dose, mg.	Rate Constants, hr.$^{-1}$ k_{12}	k_{21}	k_{13}	Volume of Distribution, V_1
1	250	1.16	1.64	0.46	14.8
2	250	2.47	1.74	1.07	7.7
3	250	0.84	1.07	0.53	12.4
4	250	0.68	1.01	0.52	16.0
5	250	0.88	1.83	0.51	17.5
6	250	0.32	1.19	0.46	16.6
7	250	0.85	1.25	0.57	14.4
8	250	0.81	1.03	0.68	11.1
9	500	0.36	0.99	0.45	14.6
10	500	0.70	0.84	0.61	10.1
11	500	1.24	1.26	0.69	10.5
12	500	0.93	0.87	0.72	11.0
13	500	1.92	1.34	0.91	7.4
14	500	0.32	0.64	0.46	16.5
Averages					
(1–8)	250	1.00	1.34	0.60	13.8
(9–14)	500	0.91	0.99	0.64	11.7
(1–14)	—	0.96	1.19	0.62	12.9

Study 4—Renal clearances were determined by a conventional method. Six subjects received single-loading doses of 250 mg. as rapid injections; constant infusions began at the 2nd hour and terminated at the 4th hour. The rates of infusion were individually calculated with the intent of maintaining constant serum levels of 4–5 mcg./ml. Serum samples were taken at 0, 0.25, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 hr.; urine samples were collected fractionally at 0–2, 2–3, 3–4, 4–6, 6–8, 8–12, and 12–24 hr.

TWO-COMPARTMENT OPEN MODEL

Sufficient blood sample points are available to permit an attempt at compartmental analysis. The data appear to be adequately described by the two-compartment open model, which is schematically represented in Scheme I, where k_{12}, k_{21}, and k_{13} are first-order rate constants for the designated processes; and V_1 is the apparent volume of distribution for the central compartment, of which blood serum is considered to be a part. The appropriate differential equations for this model and their solutions are well known (11, 12). Only the final expressions describing the time course of events for the central and urine compartments are repeated here to facilitate subsequent discussion:



Scheme I

$$C_1(t) = \frac{D}{V_1}\left[\frac{(k_{21} - \alpha)e^{-\alpha t}}{(\beta - \alpha)} + \frac{(k_{21} - \beta)e^{-\beta t}}{(\alpha - \beta)}\right] \quad \text{(Eq. 1)}$$

and

$$X_3(t) = k_{13}fD\left[\frac{(k_{21} - \alpha)(1 - e^{-\alpha t})}{\alpha(\beta - \alpha)} + \frac{(k_{21} - \beta)(1 - e^{-\beta t})}{\beta(\alpha - \beta)}\right] \quad \text{(Eq. 2)}$$

where α and β are the hybrid disposition constants such that $\alpha\beta = k_{21}k_{13}$ and $\alpha + \beta = k_{12} + k_{21} + k_{13}$; D is the dose injected; $C_1(t)$ is the concentration of antimicrobial activity in the central compartment (serum concentration) at time t; $X_3(t)$ is the cumulative amount of activity in the urine up to time t; and f is the fraction of the dose that is ultimately excreted in the urine.

Pharmacokinetic parameters depicted in the model for individual subjects were extracted from serum concentration data by the method of residuals and calculated with the aid of Eq. 1. These are shown in Table I. Urinary excretion data from the same subjects provided an internal verification on the consistency of the proposed model. Accordingly, individual urinary excretion profiles were gen-

Table II—Cumulative Urinary Excretion of Phosphonomycin following Single Injections, Observed *versus* Predicted[a]

Subject	Dose, mg.	Source	Hours: 2	4	8	12	24	36
			Milligrams Excreted					
1	250	Predicted	83.7	125.7	166.6	181.7	190.0	—
		Observed	110.2	136.7	168.5	183.3	190.5	—
2	250	Predicted	151.0	204.3	240.3	248.2	250.4	—
		Observed	151.8	199.3	235.6	245.2	256.5	—
3	250	Predicted	127.7	182.5	234.0	252.2	261.8	—
		Observed	144.0	188.2	238.1	253.1	262.5	—
4	250	Predicted	129.2	182.2	227.6	241.8	248.2	—
		Observed	134.6	184.4	223.9	235.8	248.5	—
5	250	Predicted	135.1	196.3	245.5	259.2	264.3	
		Observed	169.6	203.8	250.0	261.0	264.5	—
6	250	Predicted	136.2	193.5	237.4	248.9	252.7	—
		Observed	160.2	212.3	232.7	247.1	252.9	—
7	250	Predicted	117.7	166.5	207.7	220.2	224.3	—
		Observed	122.7	159.8	204.1	218.6	226.0	—
8	250	Predicted	131.8	178.2	215.9	226.5	230.5	—
		Observed	116.4	168.2	206.0	225.2	231.0	—
9	500	Predicted	246.8	353.3	441.4	468.0	478.7	479.5
		Observed	111.2	323.9	432.9	460.5	484.2	484.3
10	500	Predicted	200.1	274.1	341.0	364.0	374.7	375.4
		Observed	157.3	261.3	323.1	345.7	356.9	369.8
11	500	Predicted	245.6	343.2	425.5	450.0	460.9	461.4
		Observed	105.2	289.0	406.0	433.5	460.0	460.0
12	500	Predicted	331.6	446.6	548.9	581.8	597.4	598.0
		Observed	224.8	489.4	565.2	581.8	598.5	598.6
13	500	Predicted	246.4	336.7	410.1	430.5	437.5	438.0
		Observed	251.3	307.4	421.5	425.8	442.4	442.4
14	500	Predicted	206.7	290.8	365.6	392.5	407.8	408.2
		Observed	85.6	311.7	366.8	395.1	401.2	402.3

[a] With the aid of Eq. 2 using parameters from Table I.

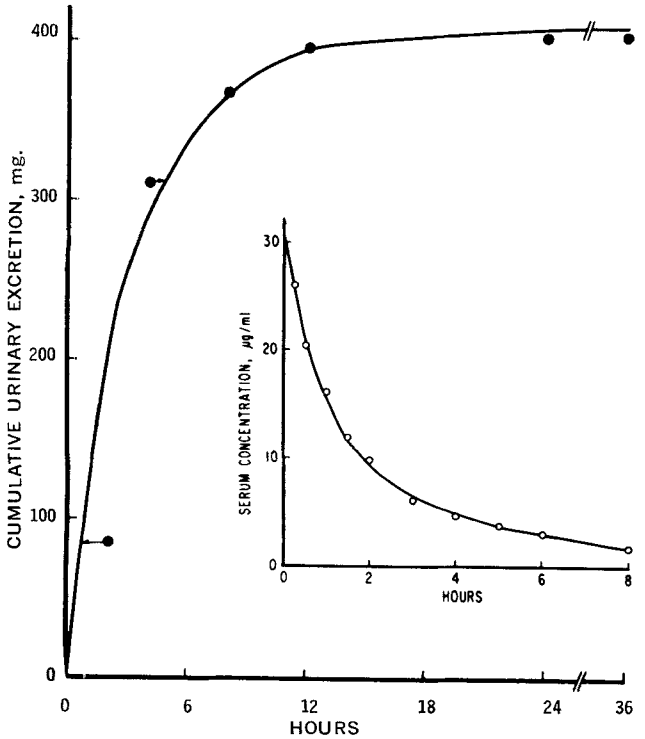


Figure 2—*Typical serum and urinary excretion profiles. Key: observed serum* (○) *and urine* (●)*; predicted* (——). *Subject 14.*

erated with Eq. 2, using parameters given in Table I. A comparison between calculated and experimentally observed urinary excretion profiles is shown in Table II. Typical serum concentration and urinary excretion profiles are also shown in Figs. 1 and 2. There is good agreement between the observed and calculated profiles except during the early time periods in the urinary excretion profiles of some individuals.

Renal and Serum Clearance—One of the seldom discussed prerequisites in the application of Eq. 2 is that renal clearance is presumed to be constant (11, 13). The single-dose data permit the evaluation of serum clearance and also of renal clearance in several ways. These are discussed in turn because their interrelationship provides some insight into the experimental situation and helps to strengthen the proposed model for phosphonomycin.

Renal clearance is usually defined as follows:

$$\dot{V}cl,r \equiv \frac{C_3 \cdot \dot{V}\mu}{C_{1,m}} = \frac{X_3(t_2) - X_3(t_1)}{(t_2 - t_1)C_{1,m}} \qquad \text{(Eq. 3)}$$

where $\dot{V}cl,r$ is the renal clearance, C_3 is the urine concentration, $\dot{V}\mu$ is the rate of urine formation, $X_3(t)$ is the cumulative amount excreted in the urine at time t, and $C_{1,m}$ is the serum concentration taken at the midpoint of the urine collection interval between t_1 and t_2. Values of $\dot{V}cl,r$ were calculated by Eq. 3 for each subject over time intervals for which the data were amenable to this treatment. They are shown in Table III. It is quite apparent that renal clearance calculated in this manner is not constant either incrementally or cumulatively.

Table III—Renal Clearance of Phosphonomycin, Milliliters per Hour, Estimated in Accordance with Eq. 3

$$\dot{V}cl,r = \frac{X_3(t_2) - X_3(t_1)}{C_{1,m}(t_2 - t_1)}$$

Subject	Dose, mg.	Urine Collection Intervals, hr. 0–2	2–4	4–8	0–4	0–8	0–12
1	250	7,344	—	3,975	5,792	8,775	7,637
2	250	10,259	—	8,820	10,831	13,328	20,437
3	250	10,919	—	6,905	7,844	10,262	11,162
4	250	9,750	—	7,601	10,975	12,169	15,113
5	250	12,852	—	8,702	9,611	13,588	16,733
6	250	10,539	—	3,913	10,406	12,118	15,842
7	250	9,158	—	8,533	9,077	10,206	14,009
8	250	8,086	—	7,875	9,347	11,198	15,640
9	500	2,940	12,972	9,400	6,921	8,590	13,234
10	500	4,184	6,755	4,681	5,834	6,732	8,732
11	500	3,507	12,416	10,446	6,752	9,759	12,900
12	500	6,811	23,211	7,584	14,919	13,853	19,395
13	500	8,786	6,936	9,834	7,182	9,579	12,235
14	500	2,750	18,842	9,497	8,027	10,047	11,437

Table IV—Renal Clearance of Phosphonomycin, Milliliters per Hour, Estimated in Accordance with Eq. 5

$$\dot{V}cl,r = \frac{X_3(t_2) - X_3(t_1)}{\int_{t_1}^{t_2} C_1(t)dt}$$

Subject	Dose, mg.	Urine Collection Intervals, hr. 0–2	2–4	4–11	11–12	12–24
1	250	6,779	3,246	4,000	5,051	4,484
2	250	8,270	7,392	8,000	11,115	41,500
3	250	7,736	5,525	6,634	5,639	6,801
4	250	8,602	7,774	7,199	6,779	16,149
5	250	11,789	5,247	8,828	7,600	6,340
6	250	9,165	7,076	3,621	9,848	11,755
7	250	7,762	5,636	8,009	8,635	10,717
8	250	6,158	7,778	7,000	12,617	9,981
9	500	2,854	12,641	7,856	6,525	13,877
10	500	3,626	9,815	5,879	4,616	5,691
11	500	2,847	12,534	9,049	7,267	17,333
12	500	6,396	21,635	6,990	4,774	9,982
13	500	5,971	3,582	9,025	1,254	12,545
14	500	2,613	16,552	4,526	6,437	2,468

A second method for the evaluation of renal clearance takes cognizance of the fact that $C_{1,m}$ in Eq. 3 should reflect the mean serum concentration for the urine collection period (14). The serum concentration at the midpoint of the interval would reflect the mean only if $C_1(t)$ varies linearly between t_1 and t_2. Such is not the case when the data appear to fit the proposed model. However, the mean serum concentration $\bar{C}_1$ can be estimated from its first noncentral moment (Law of the Mean)[2], in that

$$\bar{C}_1 = \frac{\int_{t_1}^{t_2} C_1(t)dt}{t_2 - t_1} \qquad \text{(Eq. 4)}$$

where the numerator is the area under the serum concentration *versus* time curve between t_1 and t_2. Substituting $\bar{C}_1$ as shown in Eq. 4 for $C_{1,m}$ in Eq. 3,

$$\dot{V}cl,r = \frac{X_3(t_2) - X_3(t_1)}{\int_{t_1}^{t_2} C_1(t)dt} \qquad \text{(Eq. 5)}$$

Equation 5 is in the same form as that which Wagner (15, 16) asserted to be the preferred method in calculating $\dot{V}cl,r$.

Areas under the curve over the appropriate time intervals were obtained analytically, using the integrated form of Eq. 1 and parameters from Table I. Values of $\dot{V}cl,r$ calculated in accordance with Eq. 5 are shown in Tables IV and V. Within any given subject, there is considerable variation in renal clearance from one incremental urine collection to the next (Table IV). However, when cumulative urinary excretion is considered as in Table V, this variability is dramatically reduced, and a pattern of relatively constant renal clearance values emerges as larger increments of urine collections are considered. This smoothing effect appears to be consistent with the nature of the experiment in that the body may require some time to adjust to the continuously changing serum concentrations, particularly during the early time periods when these changes are most rapid. Also, it is quite conceivable that a subject may not always be able to void completely and on time.

In comparing Tables II and V, it is evident that there is good agreement between calculated and observed urinary excretion pro-

[2] In relation to the present discussion, the required assumptions for $C_1(t)$ to be continuous for $0 \leq t \leq \infty$, differentiable for $0 < t < \infty$, and absolutely convergent appear reasonable.

$$\dot{V}cl,r = \frac{X_3(t_2) - X_3(t_1)}{\int_{t_1}^{t_2} C_1(t)dt}$$

Table V—Renal Clearance of Phosphonomycin, Milliliters per Hour, Estimated in Accordance with Eq. 5

Subject	Dose, mg.	Urine Collection Intervals, hr. 0–2	0–4	0–8	0–12	0–24	0–36	Mean, $\bar{\dot{V}}cl,r$
1	250	6,779[a]	5,597	5,205	5,192	5,161	—	5,289
2	250	8,270	8,043	8,036	8,134	8,430	—	8,182
3	250	7,736	7,072	7,415	6,879	6,876	—	7,196
4	250	8,602	8,362	8,139	8,058	8,270	—	8,286
5	250	11,789[a]	9,754	9,568	9,465	9,404	—	9,548
6	250	9,165[a]	8,546[a]	7,636	7,738	7,798	—	7,724
7	250	7,762	7,142	7,314	7,388	7,465	—	7,414
8	250	6,158	6,580	6,653	6,932	6,985	—	6,662
9	500	2,854[a]	5,807	6,215	6,233	6,406	—	6,165
10	500	3,626[a]	4,396	4,619	4,619	4,621	4,788	4,669
11	500	2,847[a]	5,598[a]	6,342	6,394	6,635	—	6,457
12	500	6,396[a]	13,095[a]	9,707	9,429	9,444	—	9,527
13	500	5,971	5,323	5,988	5,766	5,886	—	5,787
14	500	2,613[a]	6,617	6,191	6,208	6,069	—	6,271

[a] Not included in the mean.

files for times when renal clearances are constant. Furthermore, nonagreements between observed and calculated profiles are in every case accompanied by renal clearance values that deviate in the same direction. This appears to be strong evidence in support of the proposed model; when the criterion of constant clearance is satisfied, the model adequately describes the time course of events in two distinct, measurable body compartments. In any event, the apparent nonadherence to the model can be explained and, hence, does not constitute sufficient reason for rejecting the model.

While constancy in renal clearance provides a necessary criterion for modeling, a valid model must in turn provide correct estimates of pharmacokinetic parameters, particularly a tangible one like renal clearance. Therefore, if the results shown in Table V are valid, then the mean values, $\bar{\dot{V}}cl,r$, given in the last column should be reasonable estimates of renal clearance for each subject. That such indeed appears to be the case may be inferred from a comparison with the results of renal clearance estimated by the method of constant infusion (Study 4). Table VI summarizes the results of Study 4 and those obtained by the application of Eq. 5 (as shown in Table V). Inasmuch as two different sets of subjects are involved, the comparison is made on the basis of an average individual having a body surface area of 1.73 m.^{2}.

In the case of the proposed model, pharmacokinetic theory requires that

$$\text{serum clearance} \equiv \dot{V}cl,s = k_{13}V_1 \qquad \text{(Eq. 6)}$$

and

$$\text{renal clearance} \equiv \dot{V}cl,r = fk_{13}V_1 \qquad \text{(Eq. 7)}$$

Hence,

$$\frac{\dot{V}cl,r}{Vcl,s} = f \qquad \text{(Eq. 8)}$$

Table VII shows the estimates of renal clearance by Eq. 7 and of serum clearance by Eq. 6. In addition, a comparison is made between observed values of f and those obtained from the ratios of $\bar{\dot{V}}cl,r$ (Table V) to $\dot{V}cl,s$ (Eq. 6). It is seen that the calculated values of f correctly predict the observed. This may appear to be an exercise in triviality because such equality is required by the two-compartment open model. Precisely because it is required and because renal clearance values in the numerator agree with those independently determined by constant infusion, the equality in f constitutes additional support of the proposed model. In essence, this is another way of showing that the derived function for $C_1(t)$ based on the proposed model correctly predicts the incremental change in urinary excretion.

Dose–Area Relationship—If the pharmacokinetics of phosphonomycin are dose independent, one should expect that the total areas under the serum concentration curve, $\int_0^\infty C_1(t)dt$, are linearly related to the dose adjusted for differences in body weight and that the intercept of the regression line does not differ from the origin. Although only two doses were tested (at least three doses would be required to test for linearity), statistical analysis showed that the intercept of the weight adjusted regression line did not differ significantly from zero. Thus, the data do not suggest any evidence of dose dependence in the metabolism and/or the distribution of phosphonomycin at the dosage levels employed in the study.

Multiple-Dose Kinetics—One of the more important aspects of modeling is that it provides a means to predict the fate of the drug in the body and helps to devise dosage regimens to achieve and maintain the desired body levels. Hence, the ability to predict the time course of events upon repeated administration is still another test for the adequacy of the proposed model. Two types of comparison are considered. First, because every subject in Study 3 previously participated in another study from which pharmacokinetic parameters can be estimated, there is the opportunity to assess the reliability of parameters for individual subjects. Second, since dosage regimens are seldom devised for individual patients, almost never in whom the pharmacokinetic parameters are already known,

Table VI—Renal Clearance of Phosphonomycin: A Comparison of Estimates from Pharmacokinetic Analysis and Those Independently Obtained by Constant Infusion

Subject	Body Weight, kg.	Renal Clearance, ml./min./1.73 m.^{2}
1	61.4	115[a]
2	68.2	129[a]
3	61.4	111[a]
4	77.3	120[a]
5	88.6	112[a]
6	95.5	99[a]
7	90.9	109[a]
8	68.6	118[a]
9	66.4	105[a]
10	62.7	102[a]
11	66.4	105[a]
12	72.3	120[a]
13	60.0	115[a]
14	79.1	108[a]
Average (pharmacokinetic analysis)		112.8
18	86.8	84[b]
19	67.7	134[b]
20	87.7	129[b]
21	78.2	131[b]
22	66.4	106[b]
23	69.1	97[b]
Average (constant infusion)		113.5

[a] Same as $\bar{\dot{V}}cl,r$ in Table V but corrected for body surface area according to the equation: surface area = 11.0 × (body weight)$^{0.66}$ (*Reference 17*). [b] Estimated in accordance with the method described under Study 4; values taken from *Reference 8*.

Table VII—Renal and Serum Clearance of Phosphonomycin, Milliliters per Hour, and Estimation of the Fraction of Dose Excreted in the Urine

Subject	Serum Clearance[a], ml./hr.	Renal Clearance[b], ml./hr.	Fraction of Dose —Excreted in the Urine— Calculated[c]	Observed
1	6808	5188	0.777	0.762
2	8239	8453	0.993	1.026
3	6572	6900	1.095	1.050
4	8320	8270	0.996	0.994
5	8925	9443	1.070	1.058
6	7636	7728	1.012	1.012
7	8208	7420	0.903	0.904
8	7548	6974	0.883	0.924
9	6570	6366	0.938	0.969
10	6161	4559	0.758	0.740
11	7245	6665	0.891	0.920
12	7920	9480	1.203	1.197
13	6734	5960	0.859	0.885
14	7590	6102	0.826	0.804

[a] Estimated by Eq. 6. [b] Estimated by Eq. 7. [c] The ratio of $\bar{V}cl,r$ (from Table V) to $\dot{V}cl,s$ (from the second column of this table).

it is useful to determine whether the average pharmacokinetic parameters derived from a few volunteers can adequately predict the time course of events in some healthy individual.

The predicted time course of events in the central and the urine compartments for each subject were computer simulated using the appropriate sets of pharmacokinetic parameters from Table I. The calculated values are compared with those actually observed in Tables VIII (serum) and IX (urine). A typical set of serum and urine profiles is also given in Fig. 3. The results for the average subject in Tables VIII and IX constitute an attempt to compare the arithmetic average of the observed data and the simulation values based on the average parameters from Table I. The greatest deviations between calculated and observed serum levels are seen in regions where changes are most rapid, such as the 15-min. point following each dose. As with single injections, differences between observed and predicted urinary excretion tend to disappear as larger collection periods are considered. In view of the variability inherent in the bioassay, the cumulative nature of the urinary excretion profiles and minor differences in the rates of injection from dose to dose, including that from which the parameters are derived, the agreement obtained individually and on the average appears quite satisfactory.

DISCUSSION

Plasma or serum concentration data are generally preferred for the purpose of pharmacokinetic modeling. Among other considerations, this compartment is closest to the site of absorption and is readily accessible, provided the drug or metabolite(s) can be quantitatively detected therein. This is especially true in the case of intravenous administration. The urine is the body compartment that is perhaps even more conveniently accessible from the experimental viewpoint. However, urine is at least one more compartment removed from the source of input and is therefore somewhat less sensitive to perturbations. Rapid changes in drug distribution and metabolism require frequent sampling which, in the case of urine, calls for highly cooperative subjects whose ability to void on command and to retain bladder contents until a given hour to ensure complete sampling without catheterization may be less than ideal. Even among the most cooperative, there exists an irreducible minimum in the time between samples, and the subject must be able to void completely each time. Either by choice or by necessity, however, more elegant pharmacokinetic analyses have been performed on urinary excretion profiles since the pioneering studies of Nelson and his coworkers (13, 18–20).

When both serum and urine data are available, as they are in this case, there is the opportunity for immediate verification as to the adequacy of any proposed pharmacokinetic model. The additional experimental work involved appears to be disproportionately meager compared with the information so gained. Subsequent discussions presume that the evidence set forth in previous sections is sufficient to establish the two-compartment open model as adequate for the disposition of phosphonomycin following intravenous administration.

Discrepancies between predicted and observed urinary excretion, when they occur, are mostly confined to the first one or two urine collection periods. Possible causes of such nonagreement are numerous; some may be physiologic, others experimental. Variation in urine pH can have profound effects on urinary excretion (21, 22), but it is most unlikely in the case of phosphonomycin with pKa's of 1.5 and 6.4. Under physiologic conditions, phosphonomycin exists as the monovalent and the divalent anions in the urine. Inasmuch as drug concentrations are measured in terms of antimicrobial activity, it is conceivable that products of metabolism may be more or

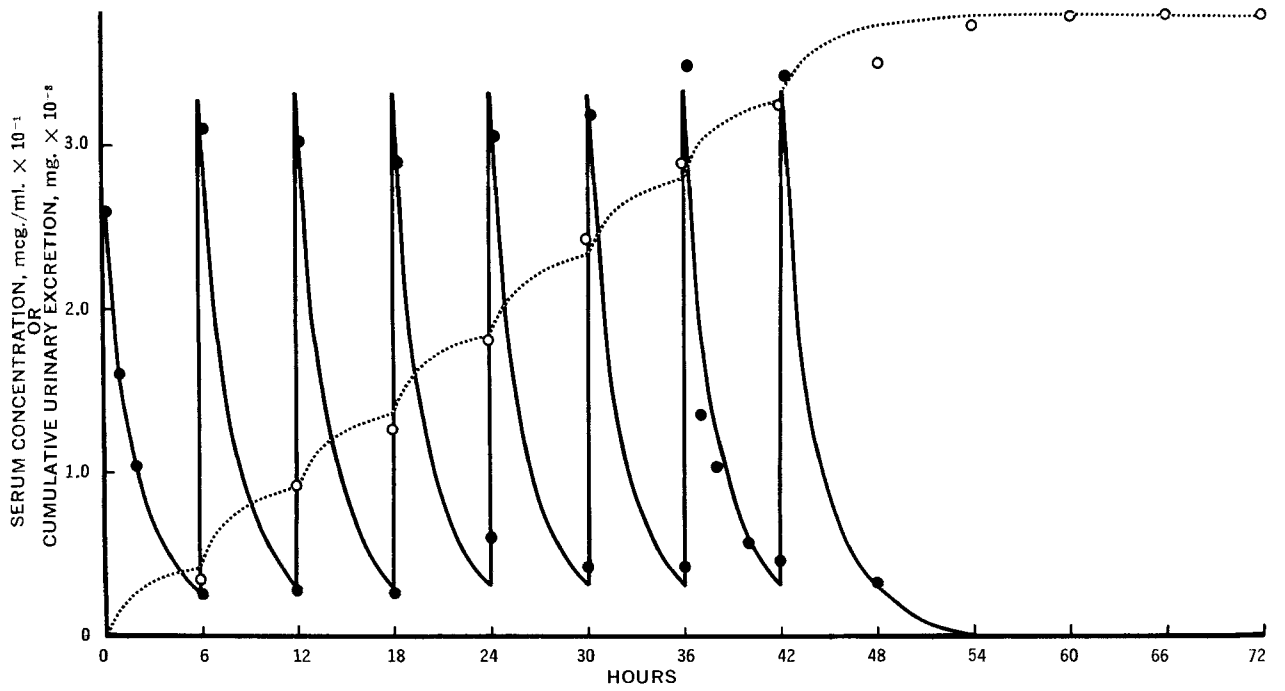


Figure 3—*Typical serum and urinary profiles of phosphonomycin on repeated intravenous injections of 500 mg. every 6 hr. Key: observed serum* (●) *and urine* (○)*; predicted serum* (——) *and urine* (- - -). *Subject 6.*

Table VIII—Observed and Predicted Serum Levels following Repetitive Administration of Phosphonomycin, 500 mg., Every 6 Hours

Subject	Source	Time from the First Dose, hr.																		
		0.25	1	2	6	6.25	12	12.25	18	18.25	24	24.25	30	30.25	36	36.25	37	38	40	42	42.25	48
		Serum Concentration, mcg./ml.																				
6	Simulated[a]	25.0	16.1	10.4	2.5	27.6	2.8	27.6	2.9	27.7	2.9	27.7	2.9	27.7	2.9	27.7	18.1	11.8	5.8	2.9	27.7	2.9
	Observed	26.0	13.7	9.0	7.0	31.0	3.2	30.3	2.7	29.0	6.0	30.6	4.3	31.9	4.3	34.8	13.6	10.4	5.7	4.6	34.2	3.3
10	Simulated[a]	36.4	18.2	10.9	3.4	39.5	4.1	40.2	4.2	40.3	4.2	40.3	4.2	40.3	4.2	40.3	21.4	13.3	7.3	4.2	40.3	4.2
	Observed	31.0	15.0	8.2	2.6	37.2	3.1	34.4	2.6	35.4	4.4	32.5	4.0	43.7	3.6	44.5	20.7	12.9	6.8	4.1	38.6	2.8
13	Simulated[a]	36.4	15.3	10.4	2.9	39.1	3.4	39.5	3.4	39.6	3.4	39.6	3.4	39.6	3.4	39.6	17.8	12.2	6.5	3.4	39.6	3.4
	Observed	30.6	15.0	10.6	3.1	34.1	3.4	35.7	4.6	34.4	4.1	34.1	5.0	34.1	5.0	42.0	24.9	15.0	7.6	4.9	37.0	2.9
15	Simulated[b]	38.2	17.5	11.8	3.8	41.8	4.6	42.4	4.7	42.6	4.7	42.6	4.7	42.6	4.7	42.6	21.1	14.6	8.3	4.7	42.6	4.7
	Observed	31.6	17.6	10.4	2.9	37.9	2.5	38.1	3.5	32.6	5.0	35.3	3.8	42.0	3.6	34.2	20.0	12.4	5.7	3.8	40.4	2.2
16	Simulated[c]	28.0	18.0	11.3	2.9	30.7	3.4	31.1	3.1	31.2	3.4	31.2	3.4	31.2	3.4	31.2	20.6	13.2	6.5	3.4	31.2	3.4
	Observed	23.6	10.2	6.8	1.8	27.8	1.9	25.8	1.6	30.9	3.6	33.5	2.7	29.5	2.7	29.5	13.6	8.4	5.2	2.7	30.0	2.1
17	Simulated[d]	26.3	15.8	9.7	2.6	28.7	3.1	29.1	3.1	29.2	3.1	29.2	3.1	29.2	3.1	29.2	18.1	11.4	5.8	3.1	29.2	3.1
	Observed	25.0	13.3	8.8	2.5	30.5	2.9	29.3	3.2	33.0	5.3	36.6	4.3	34.5	3.9	30.8	18.1	12.9	6.2	4.0	28.6	3.8
Average	Simulated[e]	27.1	13.7	8.9	2.7	29.5	3.1	30.0	3.2	30.0	3.2	30.0	3.2	30.0	3.2	30.0	16.1	10.7	5.8	3.2	30.0	3.2
	Simulated[f]	29.8	14.3	8.8	2.7	32.3	3.1	32.7	3.2	32.8	3.2	32.8	3.2	32.8	3.2	32.8	16.7	10.6	5.8	3.2	32.8	3.2
	Observed	28.0	14.1	9.0	3.3	33.1	2.8	32.2	2.9	32.8	4.8	33.8	3.9	35.9	3.9	36.0	18.5	12.0	6.2	4.0	34.8	2.9

[a] Parameters from Table I. [b] $k_{12} = 1.39$; $k_{21} = 1.20$; $k_{13} = 0.70$; $V_1 = 8.21$. [c] $k_{12} = 0.57$; $k_{21} = 1.14$; $k_{13} = 0.52$; $V_1 = 12.69$. [d] $k_{12} = 0.36$; $k_{21} = 0.84$; $K_{13} = 0.50$; $V_1 = 14.18$. [e] Average parameters for all subjects in Table I. [f] Average parameters for all subjects receiving 500 mg.

less active than the parent compound. However, there is no evidence that phosphonomycin is metabolized either in man or in animals. The possibility that there is variable and incomplete distribution in the body fluids during the early time periods is at least in qualitative agreement with the data. Since urinary recoveries depended on the cooperation of the subjects to void voluntarily on schedule as well as completely emptying their bladders, some error may have been introduced in the collections.

That these factors may be major causes of the discrepancies can be supported in several ways. Renal clearance values for each incremental urine collection period are not constant (Table IV). Constant and apparently correct values of renal clearance can be achieved by considering larger increments in a cumulative fashion. Since each cumulative increment under consideration also contains contributions from all preceding ones, correct estimates of $\dot{V}cl,r$ are possible only if the total amount excreted up to a given time is also estimated correctly. Hence, if cumulative amounts are correct but apparent deviations exist prior to that time, such deviations must be assigned to errors in timekeeping. These arguments suggest that discrepancies between observed and calculated urinary profiles should be considered as horizontal deviations rather than vertical ones (arrows in Figs. 1 and 2).

It would have been physiologically more realistic to take cognizance of the fact that some finite lag time should be incorporated into the urinary excretion profile[3]. The magnitude of this lag is estimated to be in the order of 2 or 3 min. and is usually experimentally determined. Inasmuch as the first urine sample point is at 120 min., the present data do not lend themselves to an estimation of the lag parameter with any degree of certainty. It should be noted, however, that changes attendant to a 2- or 3-min. displacement of the urinary excretion profiles would be minor in relation to the overall experimental error and, hence, should not materially influence the interpretations attempted herein.

The application of Eq. 5 requires further elaboration. The Law of the Mean guarantees that the value of C_1 used to estimate $\dot{V}cl,r$ is that of the mean serum concentration over the urine collection interval under consideration; thus, Eq. 5 is conceptually more acceptable than Eq. 3 in estimating renal clearance. In fact, Eq. 3 can be thought of as a special case of Eq. 5 in which $C_1(t)$ is linear between t_1 and t_2; hence, there is the need to achieve constant serum levels in the conventional methods of renal clearance determinations. Experimental errors notwithstanding, Eq. 5 leads to a correct estimate of $\dot{V}cl,r$ only when the denominator term can be properly estimated. $\int_{t_1}^{t_2} C_1(t)dt$ is the area under the serum concentration curve between t_1 and t_2; in practice, it may be estimated in a variety of ways, analytically if $C_1(t)$ is known, by a planimeter, by weighing, by the trapezoidal rule or Simpson's rules, *etc.* With respect to data presently under discussion, any of these methods of area estimation will lead to the same pattern for values of $\dot{V}cl,r$ when calculated by Eq. 5. However, not the same $\dot{V}cl,r$ values are achieved, and, therefore, they are not equally satisfactory as estimates of renal clearance. Hence, the applicability of Eq. 5 is largely dependent on the accuracy with which $\int_{t_1}^{t_2} C_1(t)dt$ can be estimated. The user must exercise judgement as to the adequacy of the method selected to estimate the area. It must be noted also that Eq 5. retains its present meaning only if the criteria of continuity and differentiability for $C_1(t)$ are satisfied. Otherwise, there is no assurance that the serum concentration implicit in Eq. 5 corresponds to that of the mean over the urine collection period. Consequently, when zero-order absorption, saturable process(es), or repetitive dosing is involved or suspected, there would be region(s) of $C(t)$ over which Eq. 5 should be applied only with due caution.

As in the case of spectinomycin (23), the foregoing seems to suggest that where it is safe and experimentally feasible, renal clearance can be estimated from a single rapid intravenous injection. In theory at least, this appears to be ideal in cases where an adequate model can be deduced from the data. Whenever self-consistency in the model between plasma (serum) and urine compartments can be demonstrated, a reasonable estimate of renal clearance would emerge. This is apparently the case with phosphonomycin, but the general applicability remains to be demonstrated, particularly with examples representing more complex mechanisms of excretion (24–28). There are obvious advantages

[3] The authors are indebted to Dr. J. G. Wagner and Dr. G. Levy for helpful discussions on this point.

Table IX—Observed and Predicted Urinary Excretion Profiles following Repetitive Administration of Phosphonomycin, 500 mg., Every 6 Hours

Subject	Source	Time from the First Dose, hr. 6	12	18	24	30	36	42	48	54	60	66	72
		Cumulative Urinary Excretion, mg.											
6	Simulated[a]	431	901	1375	1850	2324	2800	3274	3750	3804	3811	3812	3812
	Observed	362	942	1284	1834	2442	2896	3257	3505	3736	3784	3798	3801
10	Simulated[a]	386	823	1270	1719	2167	2616	3065	3514	3582	3582	3589	3490
	Observed	388	813	1317	1767	2170	2544	3011	3467	3561	3570	3586	3590
13	Simulated[a]	301	651	1008	1367	1725	2084	2442	2801	2846	2852	2853	2854
	Observed	183	400	802	1284	1832	2298	2326	2774	2850	2865	2866	2868
15	Simulated[b]	403	874	1358	1846	2333	2820	3308	3795	3872	3887	3890	3890
	Observed	214	647	1224	1767	2120	2676	3350	3824	3880	3895	3901	3901
16	Simulated[c]	401	838	1282	1727	2172	2617	3062	3507	3565	3574	3576	3576
	Observed	421	889	1274	1869	2211	2521	2978	3503	3539	3552	3555	3561
17	Simulated[d]	352	737	1128	1520	1912	2305	2698	3090	3143	3152	3153	3154
	Observed	120	532	918	1377	1824	2116	2554	3044	3112	3130	3136	3140
Average	Simulated[e]	379	804	1237	1671	2106	2540	2975	3409	3471	3481	3483	3483
	Simulated[f]	376	800	1233	1668	2102	2537	2971	3406	3468	3478	3481	3481
	Observed	281	704	1137	1650	2100	2508	2913	3353	3447	3468	3474	3477

[a–f] Footnotes same as those in Table VII.

to such a procedure, because it would eliminate the need to maintain constant serum levels and the potential sources of experimental error attendant thereto. It would undoubtedly be more attractive to the patient because he should be able to remain ambulatory except for sampling times. The elimination half-life and the extent of biotransformation are seen to be determining factors in considering the attractiveness of this alternative procedure. A drug that is extensively metabolized or slowly excreted in the urine is obviously not a good candidate.

Finally, words like valid, correct, adequate, proper, *etc.* have been used throughout. They are to be interpreted only in relation to this report which is based on data presently available. The authors recognize that modeling is only an approximation to the actual physical situation; refinements are always possible and are to be sought as new data are developed.

SUMMARY AND CONCLUSIONS

The two-compartment open model adequately describes the time course of events of phosphonomycin in the central and urine compartments following intravenous administration in man. Apparent inconsistencies in the proposed model are in every case accompanied by deviations from constant renal clearance. Consideration of likely causes strongly suggests that such deviations are attributed to the experimental design which relied on voluntary voiding of urine samples and the inability of the subjects to cooperate completely in performance as to completeness and time of voiding.

The adequacy of the model is further demonstrated in several ways. First, the model is shown to be adequate at two dose levels (250 and 500 mg.). Second, areas under the serum concentration curve are linearly related to dose, with an intercept not different from zero. Third, pharmacokinetic parameters derived for individual subjects satisfactorily predict the time course of events in the central and urine compartments of the same subjects following repeated administration. Average parameters also satisfactorily predict average levels. Fourth, evaluations of self-consistency of the proposed model between serum and urine lead to estimates of renal clearance in agreement with those obtained by constant infusion. Thus, a firm pharmacokinetic base has been established for the disposition of phosphonomycin in man. This is necessary to the evaluation and the understanding of the kinetics of absorption, which will be reported in a future communication.

In the process of evaluating the data, the possibility also has been suggested for the use of single intravenous injections as a potential alternate to conventional methods for the determination of renal clearance. This may be viewed as a useful by-product of pharmacokinetic analysis; it is apparently also practical in the case of phosphonomycin. The applicability of the integral method (Eq. 5) for calculating renal clearances was illustrated and discussed. Important limitations in its use are: (*a*) the conformance of the serum concentration function with the Law of the Mean requirements for continuity and differentiability, and (*b*) the accuracy in the area under the serum curve estimation.

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Stereochemical Investigations of Local Anesthetic Action

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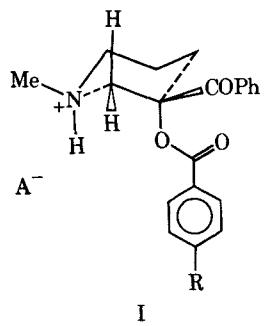
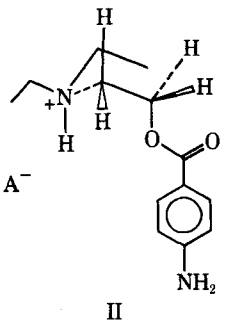
Abstract □ The toxicities and local anesthetic activities in a variety of assay systems are reported for a series of 1-alkyl-3-benzoyl-3-acyloxypiperidines. In solution, the procaine analog, XI, is conformationally more homogeneous than procaine about the 4-atom aminoethoxy unit and is 8 times more active and 16 times more toxic than procaine on a molar basis. This suggests that the preferred *gauche*-conformation of XI has positive biological significance for local anesthetic receptors on one hand and for CNS and/or cardiac receptors on the other hand, although this stereochemical feature discriminates in favor of CNS and/or cardiac receptors.

Keyphrases □ Anesthetics, local—conformation–activity relationship □ Stereochemical aspects—local anesthetic activity □ 1-Alkyl-3-benzoyl-3-acyloxypiperidines—synthesis □ Pharmacological screening—1-alkyl-3-benzoyl-3-acyloxypiperidines □ IR spectrophotometry—structure □ Polarimetry—conformation

The antipodal and diastereoisomeric potency and toxicity ratios of amino ester local anesthetics vary with the pharmacological tests used in their evaluation, but they never exceed 8 : 1 (1–5). Recent studies (6–9) on the synthesis and local anesthetic activity of asymmetric 1-methyl-3-benzoyl-3-chloropiperidine and 1-methyl-3-benzoyl-3-acyloxypiperidines (I) prompted a more rigorous pharmacological evaluation and comparison of this prototype with the chemically analogous but conformationally less restricted drug, procaine (II), in order to explore the potential relevance of conformational



I



II

restraints (*cf.*, 10, 11) about the 4-atom aminoethoxy unit to potency and/or selectivity. While this investigation is restricted to ethanolamine esters, it is of interest that conformational effects have been studied in the propanolamine series (12).

EXPERIMENTAL[1]

Chemistry

All melting points were obtained in a Hershberg-type (13) silicone (550-Dow)-filled melting-point apparatus equipped with Anschutz full-immersion thermometers and are uncorrected. The samples were placed in the silicone bath 10° below the reported melting points and heated at a rate of 1–2°/min.

Specific rotations were determined with a Zeiss 0.01° polarimeter in a modified (14) 2-dm., 2-ml. syringe-filling polarimeter tube.

IR spectra were run with a Perkin-Elmer 421 double-grating spectrophotometer as mulls in mineral oil between NaCl plates. Assignment of absorption bands, believed accurate to within ±5 $cm.^{-1}$, were made by analogy with reported values (15).

Petroleum ether refers to the fraction boiling from 30–60°. Solutions of free amines in apolar solvents were clarified with activated charcoal[2] and dried simultaneously with anhydrous sodium sulfate; they were then filtered through sintered glass. Solvents were evaporated in a water bath under reduced pressure. All base washings were performed with saturated aqueous $NaHCO_3$. All acid washings were performed with 3 *N* HCl. Unless otherwise stated, the HCl salts were prepared in Et_2O using HCl gas, dried, and recrystallized.

(+)-1-Methyl-3-benzoyl-3-benzoyloxypiperidine (III) Hydrochloride—To 13.0 g. (59.3 mmoles) of IV, (+)-1-methyl-3-benzoyl-3-hydroxypiperidine, m.p. 72.5–73.0°, $[\alpha]_D^{25}$ (absolute EtOH) +11.4 ± 0.3° (c 5.00) [lit. (10, 11) 72.5–73.0°, +11.4°] in 100 ml. of pyridine was added 18 g. (80 mmoles) of benzoic anhydride. The solution was allowed to reflux for 24 hr. After evaporation of the solvent, the residue was mixed with 100 ml. of 3 *N* HCl. The mixture was washed with $HCCl_3$, made basic with Na_2CO_3, and extracted with $HCCl_3$. The extract was dried, filtered, and evaporated to give an oil which, on clarification in and crystallization from petroleum ether, afforded 9.28 g. (28.7 mmoles, 48%) of III: m.p. 65–66°; $[\alpha]_D^{28}$ (absolute EtOH) +61.4 ± 0.4° (c 9.00); IR 1705 $cm.^{-1}$ (C=O, aromatic ester), 1676 $cm.^{-1}$ (C=O, aromatic ketone), no OH band in the 3500-$cm.^{-1}$ region.

Anal.—Calcd. for $C_{20}H_{21}NO_3$: C, 74.3; H, 6.55; N, 4.33. Found: C, 74.0; H, 6.62; N, 4.14.

The HCl salt was crystallized from 2-PrOH[3]: m.p. 234–235°.

[1] Elemental analyses were performed by Weiler and Strauss, Oxford, England.
[2] American Norit Co.
[3] 2-PrOH = isopropyl alcohol.