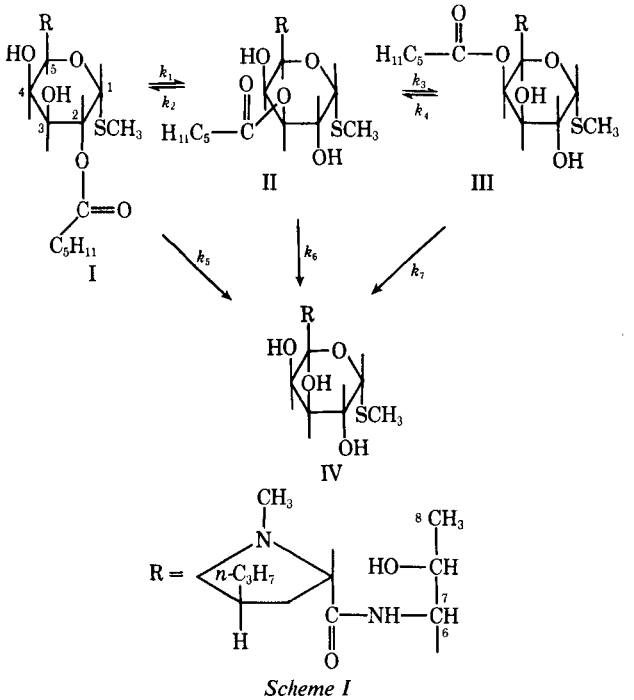
Kinetics of Alkaline Isomerization and Hydrolysis of Lincomycin Monoesters

T. O. OESTERLING▲ and C. M. METZLER

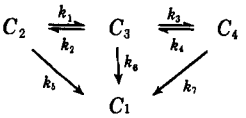
Abstract □ In alkaline media the 2-, 3-, and 4-monohexanoates of lincomycin rapidly isomerize, each entering into facile equilibrium with the other two, accompanied by hydrolysis of each species to lincomycin. The concentration–time curves of the three esters and of lincomycin as observed in a single experiment provide information about the kinetic rate constants. By using a digital computer and a nonlinear estimation program, it is possible to estimate simultaneously the four isomerization rate constants and the three hydrolysis rate constants. Estimates of the seven rate constants agree satisfactorily when either the 2-, 3-, or 4-hexanoate is the starting material, providing evidence that the postulated mechanism is correct. Acyl migration between the *cis* 3-equatorial and 4-axial hydroxyl groups of lincomycin occurs more readily than between the *trans* diequatorial 2- and 3-hydroxyl groups. In general, acyl migration occurs faster than hydrolysis where the slowest migration step is about an order of magnitude greater than the fastest hydrolysis step. The fourth possible monoester, lincomycin-7-hexanoate, undergoes straightforward hydrolysis in alkali to lincomycin and hexanoic acid.

Keyphrases □ Lincomycin monoesters—kinetics of alkaline isomerization and hydrolysis □ Isomerization rate constants—2-, 3-, and 4-monohexanoates of lincomycin, alkaline media □ Hydrolysis rate constants—2-, 3-, and 4-monohexanoates of lincomycin, alkaline media

The synthesis and biological activities of lincomycin 2-, 3-, 4-, and 7-monoesters were reported by Morozowich, Sinkula, and their coworkers (1–4). These compounds were prepared as part of a study to improve the pharmaceutical and/or biological properties of the parent drug through *in vivo* reversible derivatives.



Scheme I



Scheme II

A previous report presented qualitative information regarding the relative stabilities of the four monohexanoates and also contained chemical data gathered to establish the mechanism of degradation in acid and base. In alkaline media, monoesters of lincomycin at the 2-, 3-, and 4-positions rapidly isomerize, each entering into facile equilibrium with the other two, accompanied by hydrolysis of each species to lincomycin (Scheme I). Lincomycin-7-hexanoate undergoes straightforward hydrolysis to lincomycin and hexanoic acid under both acidic and alkaline conditions (5).

The present study was undertaken to determine the magnitude of the seven rate constants involved in the reaction shown in Scheme I. The method involved reacting each of the three monoesters under identical conditions at high pH and determining the concentration of each species as a function of time by GLC assay. For each experiment, the concentration–time data of the three esters and of lincomycin were fitted by the method of least squares, with an iterative nonlinear program and a digital computer, to the rate equations representing Scheme I to obtain k_1 through k_7. Agreement between sets of rate constants obtained by starting with each monoester could then be interpreted as support of the proposed mechanism. Knowledge of the magnitude of the specific rate constants provided basic stability information, which was helpful in synthetic and formulation studies.

EXPERIMENTAL

Kinetic Studies—A solution of lincomycin monoester in methyl alcohol (analytical reagent) at room temperature was added to an equal volume of 0.2 *M*, pH 9.5, carbonate buffer whose temperature was between 5 and 10°. After mixing, the temperature of the resulting solution was 25°; to maintain this temperature, the reaction mixture was placed in a 25° constant-temperature bath. At appropriate times, 1.0-ml. samples were withdrawn and added to 100 μl. of glacial acetic acid to quench the reaction. Samples were then freeze dried, and the residue was assayed by the procedure reported previously (5).

Estimation of Rate Constants—Let lincomycin and its esters be represented by the following symbols: C_1 = lincomycin, C_2 = lincomycin-2-hexanoate, C_3 = lincomycin-3-hexanoate, and C_4 = lincomycin-4-hexanoate. Then Scheme I may be represented as Scheme II.

By assuming first-order rate constants, the concentrations of the four compounds can be described by the system of differential equations:

$$\frac{d[C_1]}{dt} = k_5[C_2] + k_6[C_3] + k_7[C_4] \qquad \text{(Eq. 1)}$$

Table I—Species Concentrations following Degradation of Lincomycin-2-hexanoate in 50% Methanol–50% 0.2 *M*, pH 9.5, Carbonate Buffer at 25°

Minutes (t)	Moles per Milliliter of Reaction Mixture × 10^6				
	Lincomycin (C_1,t)	Lincomycin-4-hexanoate (C_4,t)	Lincomycin-3-hexanoate (C_3,t)	Lincomycin-2-hexanoate (C_2,t)	Total
0.45	0.22	0.00	0.00	5.45	5.67
3	0.62	0.72	0.60	3.90	5.84
6	1.00	1.05	0.95	3.35	6.35
9	1.38	1.38	1.10	2.35	6.21
12	1.66	1.52	1.15	1.89	6.22
15	1.92	1.55	1.02	1.45	5.94
18	2.00	1.35	0.95	1.12	5.42
25	2.75	1.35	1.00	0.90	6.00
30	2.92	1.18	0.70	0.73	5.53
40	3.70	0.82	0.65	0.61	5.78
50	4.40	0.68	0.45	0.60	6.13
60	4.78	0.52	0.32	0.48	6.10

$$\frac{d[C_2]}{dt} = -(k_1 + k_5)[C_2] + k_2[C_3] \quad \text{(Eq. 2)}$$

$$\frac{d[C_3]}{dt} = -(k_2 + k_3 + k_6)[C_3] + k_4[C_4] + k_1[C_2] \quad \text{(Eq. 3)}$$

$$\frac{d[C_4]}{dt} = -(k_4 + k_7)[C_4] + k_3[C_3] \quad \text{(Eq. 4)}$$

For a given value of the vector of rate constants $\mathbf{k} = (k_1, \ldots, k_7)$ and initial conditions $[C_{j,0}]$, $j = 1,2,3,4$, this system of differential equations can be solved by numerical integration to give predicted concentrations at time t, designated by $[C_{j,t}]$. Let the observed concentrations at time t_i, $i = 1, \ldots, n$, be designated by $[c_{j,t_i}]$, $j = 1,2,3,4$; $i = 1, \ldots, n$. Then the estimated vector of rate constants is that vector such that the predicted concentrations using these values agree best with the observed concentrations. The criterion for best agreement is the least-squares criterion; that is, the least-squares estimate $\hat{\mathbf{k}}$ is that vector which minimizes:

$$SS(\mathbf{k}) = \sum_{j=1}^{4} \sum_{i=1}^{n} \{[C_{j,t_i}] - [c_{j,t_i}]\}^2 \quad \text{(Eq. 5)}$$

There are several methods of finding the vector $\hat{\mathbf{k}}$ which minimizes $SS(\mathbf{k})$. In this study, a modified version of the Gauss-Newton method was used (6); this method uses a locally linearized form of the model equations. The method requires a first guess of the rate vector, say $\mathbf{k}^1$; then, using the linearized equations, corrections to $\mathbf{k}^1$ are computed, thus obtaining a new rate vector $\mathbf{k}^2$. Again corrections are computed and $\mathbf{k}^3$ is obtained. This process is iterated until the difference $SS(\mathbf{k}^m) - SS(\mathbf{k}^{m+1})$ is less than some predetermined difference. When this happens, the method is said to have converged to the least-squares estimate $\hat{\mathbf{k}} = \mathbf{k}^{m+1}$. A computer program, NONLIN[1], does the computations for this estimation procedure (7). Since $SS(\mathbf{k})$ is a function of the observed concentrations of all four compounds, the program obtains estimates based on the pooled information from all of the observations in each run. Equal weighting was used since there were the same numbers, n, of observations of the concentrations of each compound, and no information on the error structure was available which would indicate any other weighting.

RESULTS AND DISCUSSION

A typical set of data showing the change in concentration of each lincomycin species with time following degradation of lincomycin-2-hexanoate appears in Table I. The total number of moles of all species present at each time corresponds to the concentration of starting material and is constant, indicating no other reactions are occurring during the time interval under consideration. Mass balance was also preserved during experiments in which lincomycin-3-hexanoate and lincomycin-4-hexanoate were the starting compounds.

[1] Available from C. M. Metzler.

Table II—Rate Constants of Isomerization and Hydrolysis of Lincomycin Monoesters in 50% Methanol–50% pH 9.5 Carbonate Buffer at 25°

	Rate Constant, min.$^{-1}$ (Mean ± 1 *SD*)		
	2-Hexanoate[a]	3-Hexanoate[b]	4-Hexanoate[c]
k_1	0.114 ± 0.028	0.107 ± 0.033	0.128 ± 0.099
k_2	0.088 ± 0.026	0.117 ± 0.073	0.098 ± 0.032
k_3	1.43 ± 0.79	1.60 ± 0.96	1.23 ± 0.43
k_4	1.03 ± 0.45	1.20 ± 0.71	0.86 ± 0.47
k_5	0.028 ± 0.004	0.045 ± 0.040	0.033 ± 0.026
k_6	0.025 ± 0.007	0.016 ± 0.014	0.037 ± 0.024
k_7	0.026 ± 0.019	0.028 ± 0.048	0.0007 ± 0.001

[a] Average of seven runs. [b] Average of three runs. [c] Average of four runs.

Figure 1 shows a plot of the data in Table I, where the circles are experimental values and the solid lines are least-squares curves obtained by computer analysis which best fit the data. Similar sets of curves and experimental values are shown in Figs. 2 and 3, where the 3- and 4-hexanoates of lincomycin were starting compounds, respectively. The fit of experimental points to the least-squares curves shown in Figs. 1–3 is typical of that observed in all studies. In some cases, it appears that a better fit to the experimental observations of an individual species might be possible, such as the lincomycin-4-hexanoate curve in Fig. 2. However, the least-squares curve of each species is restrained by the data points of the other species, and it represents the curve determined by the parameter set that best fits the experimental values for all four species in the run.

Rate constants for each of the seven transformations indicated in Scheme I, obtained when each of the lincomycin monoesters was the starting material, are shown in Table II. The relative magnitude of k_1 through k_7 is independent of the starting ester, and analysis of variance of each estimated rate constant showed no statistically significant difference due to the starting ester. On the other hand, the standard deviations of several estimates are relatively large, and a few of the hydrolysis rate constants are not consistent with the remainder of the data. For example, estimates of k_5 and k_6 when lincomycin-3-hexanoate was the starting material did not show the same relative magnitude compared to the same rate constants obtained from the 2- and 4-hexanoates, and the estimate of k_7 when lincomycin-4-hexanoate was the starting material was not consistent with the remainder of the data. The estimates of k_5 and k_6 when 3-hexanoate was the starting material are not significantly different from the other estimates of k_5 and k_6 in Table II. However, the estimate of k_7 resulting from degradation of the 4-hexanoate was definitely not compatible with the other estimates of k_5 through k_7. The reason for the low magnitude and high degree of variability in the estimation of this rate constant is unknown. One possible explanation is that the mechanism proposed in Scheme I is incorrect. However, the internal agreement of the remainder of the data in Table II and the chemical data presented elsewhere (5) are strong evidence that the proposed mechanism is correct.

Although the data in Table II lack the precision that might be obtained from more extensive studies, the quality is such that much useful information can be obtained. This information includes the determination of the relative magnitude of hydrolysis and migration processes and the determination of the relative reactivities of the three hydroxyl groups on the galactopyranoside moiety with respect to migration and hydrolysis.

The migration rate constants, k_1 through k_4, are greater than the hydrolysis rate constants, k_5 through k_7, where the slowest migration step (k_1 and k_2) is about an order of magnitude faster than the fastest hydrolysis step. Similar behavior was observed by Wolfenden *et al.* (8), where rates of acyl migration of monoglycerides were about 6000 times faster than hydrolyses under similar conditions. Because of the large difference in the magnitude of isomerization and hydrolysis rate constants, it was possible to find conditions by proper pH control where essentially only migration was occurring. Such a feature would be desirable in the lincomycin monohexanoate systems for the synthesis of positional isomers. However, since

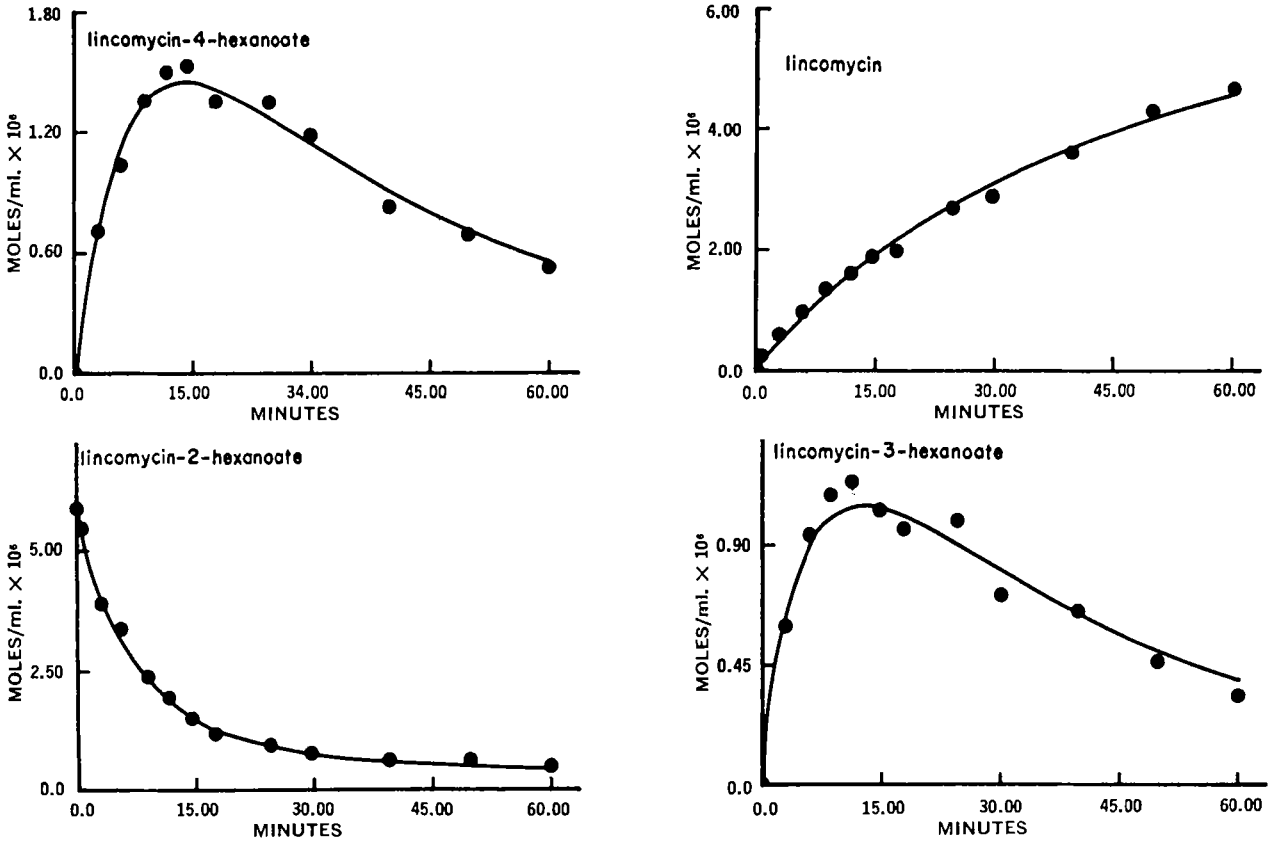


Figure 1—*Species concentration–time curves following alkaline degradation of lincomycin-2-hexanoate. Key:* ●, *experimental; and* ——, *calculated.*

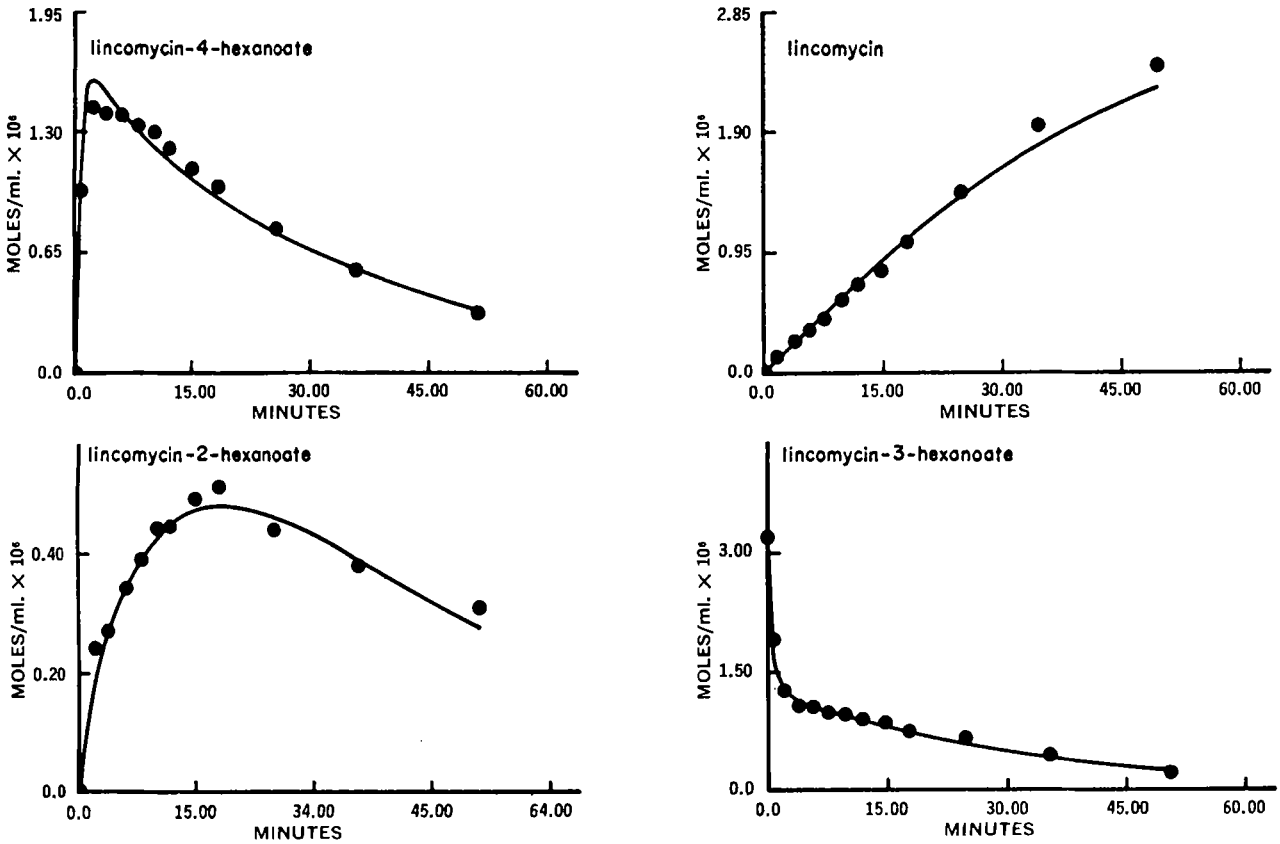


Figure 2—*Species concentration–time curves following alkaline degradation of lincomycin-3-hexanoate. Key:* ●, *experimental; and* ——, *calculated.*

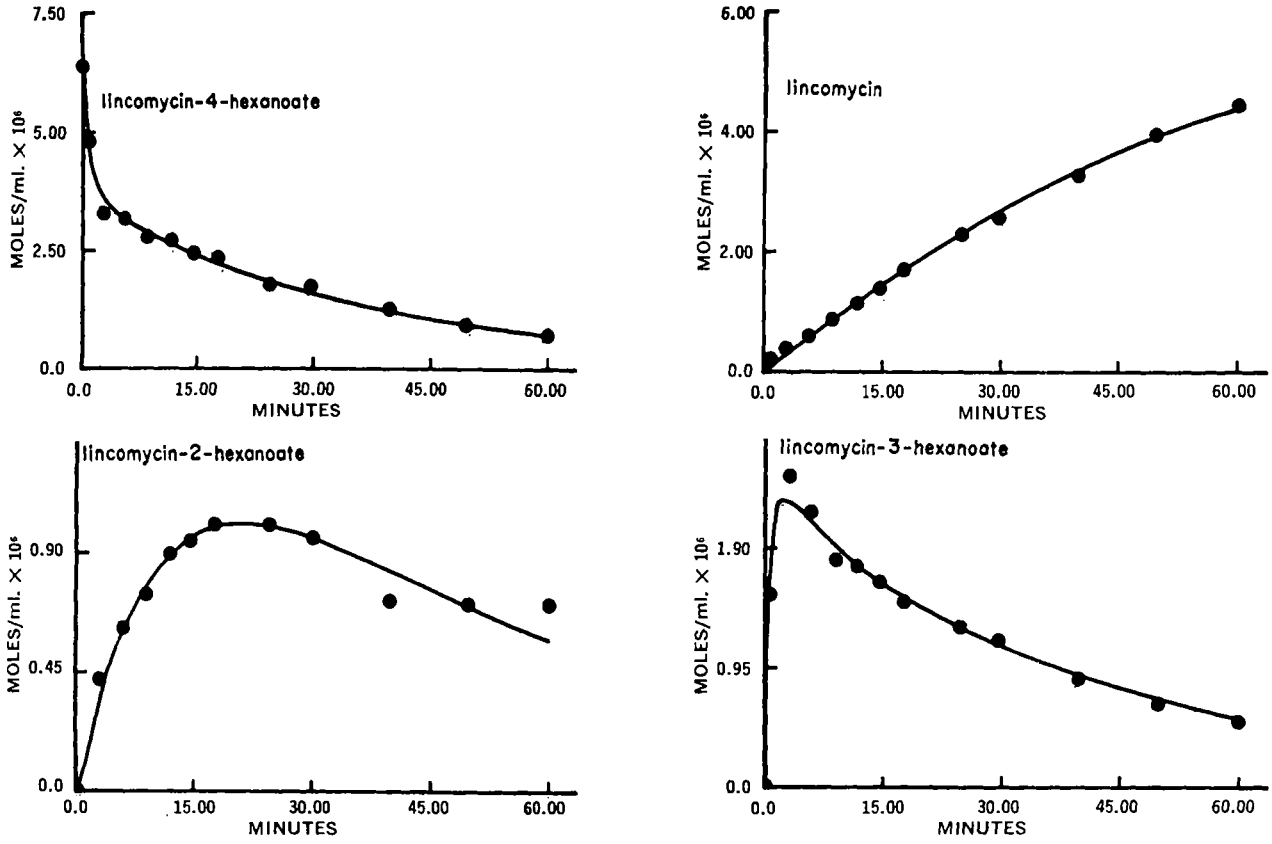
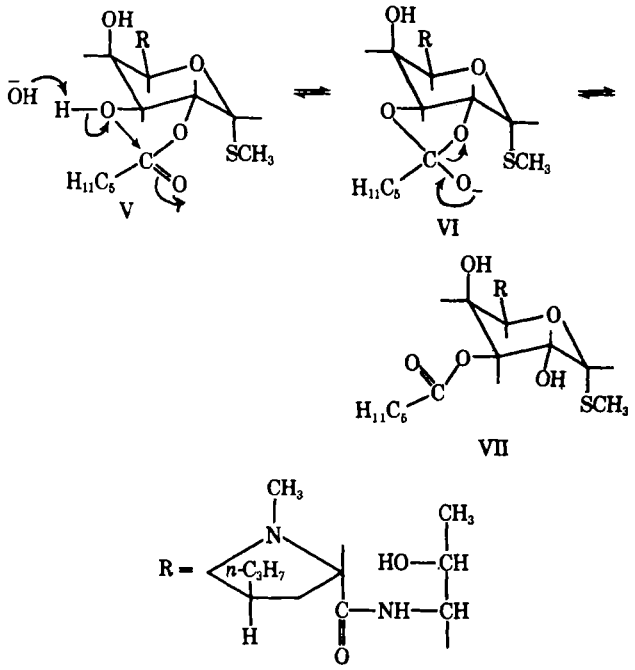


Figure 3—*Species concentration–time curves following alkaline degradation of lincomycin-4-hexanoate. Key:* ●, *experimental; and* ——, *calculated.*

migration and hydrolysis differ by only 10-fold in aqueous systems, it is not possible to isolate migration as in the glyceride systems. On the other hand, hydrolysis is minimized in nonaqueous systems, and positional isomers of lincomycin, such as the 4-hexanoate, can be obtained in high yield by acid- or base-catalyzed migration of the 3-hexanoate in *N,N*-dimethylformamide (9).

Scheme III shows the mechanism by which acyl migration from



Scheme III

the 2- to 3-position of lincomycin probably occurs. A similar process would occur in the interconversion between the 3- and 4-hexanoates of lincomycin. Migrations such as the one shown in Scheme III were extensively reported (10–13) and are thought to occur through cyclic *ortho*-acid or ester intermediates (VI) first postulated by Fischer (14). In the monohexanoate systems reported in this study, cyclic intermediates such as VI are relatively unstable since none was observed by TLC (5) or VPC, and mass balance was maintained (Table I).

The fastest isomerization processes take place in the interconversion of lincomycin-3-hexanoate and lincomycin-4-hexanoate, where k_3 and k_4 are approximately 10 times greater than k_1 and k_2. Thus, when lincomycin-3-hexanoate is the starting material, 10 moles of 4-hexanoate are formed to every mole of 2-hexanoate. This favored migration of acyl groups between positions 3 and 4 is probably due to the relative ease of formation of the cyclic intermediate involved in the process. Inspection of Dreiding models shows that the intermediate formed between positions 3 and 4 (whose hydroxyl groups are *cis*) is much less strained than the intermediate formed between the *trans*-C_2- and C_3-hydroxyl groups. Synthesis of acetals of lincomycin yields primarily the 3,4-derivative (15, 16), providing further evidence that the *cis*-hydroxyls are favored for cyclic structure formation. Also, acyl migration between vicinal hydrolysis of furanose sugars occurs more readily when the substituents are *cis* (17).

Morozowich *et al.* (9) took advantage of the favored migration between positions 3 and 4 of the galactoside moiety shown in Scheme I by synthesizing the 4-hexanoate of lincomycin and of its 7 (S)-chloro analog, clindamycin, in high yield from the more readily available 3-hexanoate. Also, migration to position 2 from position 3 can be forced by blocking the 4-position with a protective group. For example, Chittenden and Buchanan (18) prepared benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside from the 3-benzoate in 0.05 *N* NaOH in acetone. Since the favored migration pathway was blocked, they obtained high yields of the less favored C_2-isomer.

Although there is more variability in the estimates of hydrolysis relative to isomerization rate constants, the most reasonable conclusion that can be drawn from the data in Table II is that hydrolysis

of the 2-, 3-, and 4-hexanoates of lincomycin occurs at approximately the same rate. From the standpoint of configuration of the C_2, C_3, and C_4 substituents, equality of k_5 through k_7 would not be expected. In general, axial substituents are less reactive than equatorial (19) and, since the C_4 substituent of lincomycin is axial while those at C_2 and C_3 are equatorial, substituents at C_4 should be less reactive. For the case of acylation, the C_4-hydroxyl is least reactive since 2,3-diesters of lincomycin can be synthesized even in the presence of excess acylating agent (20).

However, a factor that must be considered in addition to substituent configuration with respect to hydrolysis of lincomycin-2-, 3-, and 4-hexanoates is neighboring group effects. All three galactopyranoside monoesters of lincomycin are subject to neighboring hydroxyl group facilitation of base-catalyzed hydrolysis (5, 21, 22). For example, esters at position 3 should hydrolyze faster due to the presence of the adjacent *cis*-2-hydroxyl and *trans*-4-hydroxyl groups. Hydrolysis of 2- and 4-acylates would be facilitated by the 3-hydroxyl, but neither would significantly influence the other. Hydrolysis of lincomycin-2-acylates is probably also facilitated by the neighboring *cis*-1-thiomethyl group (23). In addition to facilitation by C_3—OH, hydrolysis of lincomycin-4-hexanoate may be influenced by the hydroxyl group at C_7. Hydrogen bonding between positions 4 and 7 was demonstrated (24), and Douglas (25) obtained evidence that hydrolysis of the 4-palmitate of lincomycin is facilitated by the C_7—OH. For example, when clindamycin-2-palmitate is treated with dilute sodium hydroxide for short periods of time, much larger amounts of 4-ester remain relative to 2- and 3-palmitates, whereas approximately equal amounts of 2-, 3-, and 4-esters remain when lincomycin-2-palmitate is treated under identical conditions. Thus, clindamycin-2-palmitate, which did not contain a C_7—OH group for facilitation, apparently hydrolyzed slower than its 7-(R)-OH anomer.

Considering the relative influence of substituent configuration and neighboring group facilitation on the hydrolytic reactivity of the 2-, 3-, and 4-hexanoates of lincomycin, it appears that neighboring group effects play a dominant role. The best mechanistic interpretation consistent with the values of k_5 through k_7 in Table II is that the influence of substituent configuration is minimal due to the much larger neighboring group effects which render the three esters equally reactive.

REFERENCES

(1) W. Morozowich, A. A. Sinkula, F. A. MacKellar, and C. Lewis, to be published.

(2) A. A. Sinkula, W. Morozowich, C. Lewis, and F. A. MacKellar, *J. Pharm. Sci.*, **58,** 1389(1969).

(3) H. Morozowich, F. A. MacKellar, and C. Lewis, to be published.

(4) *Ibid.*, to be published.

(5) T. O. Oesterling, *Carbohyd. Res.*, **15,** 285(1970).

(6) H. O. Hartley, *Technometrics*, **3,** 269(1961).

(7) C. M. Metzler, "NONLIN, a computer program for parameter estimation in nonlinear situations," 1969.

(8) R. Wolfenden, D. H. Rammler, and F. Lipmann, *Biochemistry*, **3,** 329(1964).

(9) W. Morozowich, F. A. MacKellar, and C. Lewis, to be published.

(10) D. J. Hanahan, "Lipid Chemistry," Wiley, New York, N. Y., 1960, p. 194.

(11) W. Pigman, "The Carbohydrates," Academic, New York, N. Y., 1957, p. 147.

(12) J. M. Sugihara, in "Advances in Carbohydrate Chemistry," vol. 8, C. S. Hudson and M. L. Wolfrom, Eds., Academic, New York, N. Y., 1953, p. 2.

(13) E. Pascu, in "Advances in Carbohydrate Chemistry," vol. 1, W. W. Pigman and M. L. Wolfrom, Eds., Academic, New York, N. Y., 1945, p. 111.

(14) E. Fischer, *Ber.*, **53,** 1621(1920).

(15) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Amer. Chem. Soc.*, **86,** 4223(1964).

(16) M. J. Taraszka and W. Morozowich, *J. Org. Chem.*, **33,** 2349(1968).

(17) S. Tejima and H. G. Fletcher, *ibid.*, **28,** 2999(1963).

(18) G. J. F. Chittenden and J. G. Buchanan, *Carbohyd. Res.*, **11,** 379(1969).

(19) E. L. Eliel, N. L. Allinger, S. J. Augyal, and G. A. Morrison, "Conformational Analysis," Interscience, New York, N. Y., 1965, p. 269.

(20) A. A. Sinkula, W. Morozowich, and E. L. Rowe, to be published.

(21) H. B. Henbest and B. J. Lowell, *J. Chem. Soc.*, **1957,** 1965.

(22) T. C. Bruice and T. H. Fife, *J. Amer. Chem. Soc.*, **84,** 1973 (1962).

(23) W. Davis and W. C. J. Ross, *J. Chem. Soc.*, **1950,** 3056.

(24) G. Slomp and F. A. MacKellar, *J. Amer. Chem. Soc.*, **89,** 2454(1967).

(25) S. L. Douglas, The Upjohn Co., personal communication.

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▲ To whom inquiries should be directed.