Although these data clearly showed that the comparative viscosity decreased with added water as would be expected, the actual differences in viscosity between the various systems were felt to be greater than the numerical differences in the penetrometer readings. This is because the probe used for the measurements was an inverted cone and at greater penetrations, a greater cross section of the cone would contact the sample. Therefore, for greater penetration, a larger portion of the sample would support the load and prevent further penetration. A better estimate of the difference in relative viscosity of these samples would take into account this change in effective contact area of the probe with the sample. Considering this aspect, it would then be estimated that the sample with 27.5% water would be over 200 times as viscous as the sample with 48.5% water. From Fig. 14, the 5% magnesium stearate sample would therefore approximately correspond to the 27% water sample while the drug–$CaHPO_4 \cdot 2H_2O$ behaved like the 48% water sample.

The combined interpretation of Figs. 13 and 14 would suggest that magnesium stearate did reduce the water content in the powder bed and that this, in turn, yielded a wet powder blend of high viscosity. Dissolution of the drug would then be slowed due to the limited area of contact between the wet powder mass and the fluid. Dissolution would proceed by mechanical erosion, diffusion, and/or solution of drug and filler. *In vivo* it would seem these powder masses might remain intact for a considerable time if the agitation conditions are indeed low. In addition, an *in vivo* pH near neutral would favor stability of the wet powder mass.

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Hydrolysis of Lincomycin-2-phosphate and Clindamycin-2-phosphate

T. O. OESTERLING and E. L. ROWE

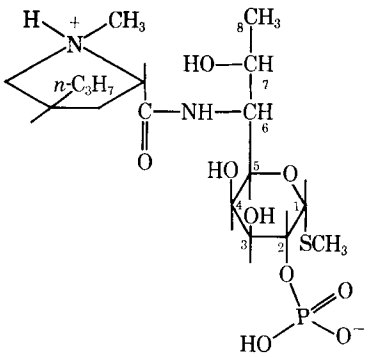
Abstract □ The aqueous stabilities of lincomycin-2-phosphate and its 7-deoxy-7(S)-chloro analog, clindamycin-2-phosphate, were studied at a variety of temperatures and pH values. The predominant degradative routes of lincomycin-2-phosphate in the pH range 1–10 are thioglycoside and phosphate ester hydrolysis, and pH-rate studies show that it is most stable at pH 6–10. Clindamycin-2-phosphate degrades by three major routes from pH 1 to 10 with phosphate ester and thioglycoside hydrolysis predominating at pH less than 6 and scission of 7(S)-Cl to form the 7(R)-OH analog predominating at pH greater than 6. The rate of 7(S)-Cl to 7(R)-OH conversion was obtained using gas chromatography to measure the disappearance of clindamycin in reaction mixtures prepared under identical conditions as clindamycin-2-phosphate reaction mixtures. Rates of phosphate ester hydrolysis of the two compounds were measured by spectrophotometric determination of the amount of inorganic phosphate formed in reaction mixtures as a function of time. Apparent first-order rate constants of phosphate ester hydrolysis as well as activation energies (32.1 kcal./mole for lincomycin-2-phosphate and 32.9 kcal./mole for clindamycin-2-phosphate) agree favorably. The rate of lincomycin-2-phosphate hydrolysis in a formulated pediatric syrup at room temperature agrees favorably with extrapolated rates from high temperature hydrolysis in simple solutions.

Keyphrases □ Lincomycin-2-PO_4—hydrolysis kinetics □ Clindamycin-2-PO_4—hydrolysis kinetics □ Stability—lincomycin-, clindamycin-2-PO_4 aqueous solutions □ pH effect—lincomycin-, clindamycin-2-PO_4 hydrolysis rates □ Colorimetric analysis—spectrophotometer

The antibiotics lincomycin and clindamycin are highly effective in the treatment of infections caused by Gram-positive organisms (1, 2) and clindamycin possesses marked antiplasmodial activity as well (3). At times it is desirable to make derivatives from compounds such as lincomycin and clindamycin in order to circumvent disadvantages such as bitter taste or poor absorption inherent in the parent molecule. The derivatives must possess the same activity as the parent compound or be rapidly reverted to parent *in vivo*. Lincomycin has a bitter taste which is difficult to mask in pediatric liquid formulations. The C_2 phosphate ester of lincomycin

was synthesized by Morozowich *et al.* (4) as a potential nonbitter derivative with the same activity as the parent compound. Clindamycin hydrochloride is poorly soluble at pH values above 6 and its C_2 phosphate ester was synthesized (5) to obtain a derivative of high water solubility near neutral pH.

The purpose of this study was to investigate the stability of lincomycin-2-phosphate (I) and clindamycin-2-phosphate (II) in aqueous solutions in order to supply basic information necessary for the successful formulation of these drugs in liquid dosage forms. The effects of pH and temperature on the rate of phosphate ester hydrolysis of lincomycin-2-phosphate and clindamycin-2-phosphate were determined. These data allowed the establishment of maximum stability conditions and the prediction of room temperature stability.



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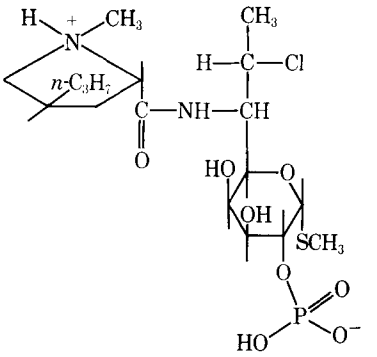

II

EXPERIMENTAL

Materials—Lincomycin-2-phosphate, clindamycin-2-phosphate, and clindamycin hydrochloride containing less than 2% impurities were supplied by the research laboratories of the Upjohn Company. All other chemicals were reagent grade.

Kinetic Studies—Lincomycin-2-phosphate—The rate of hydrolysis of lincomycin-2-phosphate was studied in the pH range 6–9 at 59.4, 69.6, 79.6, and 93.5° and in the extended pH range 0.1–10 at 90.0°. All reaction mixtures except those of less than pH 2 at 90° were prepared at the temperature of the run by adjusting the pH of 90 ml. of solution containing lincomycin-2-phosphate and sodium chloride to the desired pH by the addition of 1 *M* HCl or NaOH. After the volume was adjusted to 100 ml., the final concentration of lincomycin-2-phosphate was 0.02 *M* and that of sodium chloride was 0.1 *M*. The sodium chloride was added to minimize ionic strength effects. Reaction mixtures adjusted to pH less than 2 at 90° were prepared to contain 0.02 *M* lincomycin-2-phosphate and 0.1, 0.5, and 1 *M* HCl, respectively. These reaction mixtures did not contain sodium chloride. After preparation, 5-ml. portions of reaction mixtures were filled into ampuls, sealed, and immersed into the appropriate constant-temperature baths. Ampuls were withdrawn at appropriate times and frozen until assayed for inorganic phosphate by the procedure described below.

Clindamycin-2-phosphate—The effect of pH on the rate of phosphate ester hydrolysis of clindamycin-2-phosphate was studied in the pH range 6–9 at 90°. It was necessary to buffer clindamycin-2-phosphate reaction mixtures because, unlike lincomycin-2-phosphate, the pH drifted sharply downward as the reaction proceeded. This decrease in pH may be caused by degradation at C_7 of clindamycin-2-phosphate (6). Reaction mixtures were prepared to contain 0.02 *M* clindamycin-2-phosphate, 0.2 *M* ethylenediamine, or tris (hydroxymethyl) aminomethane[1] (tromethamine) buffers adjusted to the desired pH at the temperature of the run, and sufficient potassium chloride to adjust the ionic strength to 0.3. Aliquots of the reaction mixtures were filled into ampuls which were sealed and immersed in constant temperature baths. Ampuls were withdrawn at appropriate times and frozen until assayed for inorganic phosphate by the procedure described below.

All reaction mixtures in which phosphate ester hydrolysis was measured were prepared with freshly boiled deionized water. The pH's of the samples from all kinetic runs were measured, and a run was discarded if the pH of the final sample varied by more than 0.2 pH units from the initial sample.

Rates of phosphate ester hydrolysis were determined by least-squares analysis of log $(P_{i,\infty} - P_{i,t})$-time data, where $P_{i,\infty}$ is the molar concentration of inorganic phosphate present at completion of hydrolysis which is equivalent to the initial molar concentration of lincomycin-2-phosphate or clindamycin-2-phosphate, and $P_{i,t}$ is the molar concentration of inorganic phosphate present at any time.

A previous study (6) has indicated that in addition to phosphate ester hydrolysis at Position 2, clindamycin-2-phosphate will undergo degradation at C_7 to form the 7-(R)-OH analog in the pH range 6–9. To measure the rate of degradation at Position 7, reaction mixtures containing clindamycin hydrochloride were prepared identical to those containing clindamycin-2-phosphate, and the amount of intact clindamycin remaining with time was determined by GLC (6).

Inorganic Phosphate Assay—The method is essentially that developed for assay of free inorganic phosphate in methylprednisolone phosphate (7). The reagents used were ammonium molybdate, 2.0% solution in double-distilled water, acetate buffer prepared by dissolving 310 ml. glacial acetic acid, 49 g. anhydrous potassium acetate, and 48 mg. anhydrous cupric sulfate in double-distilled water to a total volume of 1000 ml., 2.0% ascorbic acid solution in double-distilled water, and 20% stannous chloride stock solution in concentrated hydrochloric acid. One milliliter of the stock stannous chloride solution was diluted to 100 ml. with double-distilled water just prior to use.

Stability samples were assayed for inorganic phosphate by adding 1.0-ml. aliquots of the reaction mixtures to 50 ml. of acetate buffer in 100-ml. volumetric flasks. After thorough mixing, 5.0 ml. ammonium molybdate solution, 5.0 ml. ascorbic acid solution, and 5.0 ml. of the diluted stannous chloride solution were added with mixing after each addition. The solution was adjusted to 100 ml., mixed again, and allowed to stand 30 min. The absorbance was determined at 740 mμ against a water blank using 1.0-cm. cells within a period of 3 hr. After confirming that Beer's law applied in the concentration range of interest, the concentration of inorganic phosphate was determined by comparing the absorbance of the unknown solutions with that of a standard solution.

RESULTS AND DISCUSSION

The phosphate ester moieties of lincomycin-2-phosphate and clindamycin-2-phosphate hydrolyzed by an apparent first-order process under all experimental conditions. Some typical first-order curves of lincomycin-2-phosphate hydrolysis are shown in Fig. 1.

The effects of pH and temperature on the rate of phosphate ester hydrolysis of lincomycin-2-phosphate are shown in Table I. In general the hydrolysis rate constants from reaction mixtures of similar pH were in good agreement. The discrepancy in the data from the pH 1.1 reaction mixtures at 90° may be due to the high dependency of rate constant on pH in this region. The rates of phosphate ester hydrolysis of clindamycin-2-phosphate as a function of pH and temperature are shown in Table II. Although ionic strengths are different and the clindamycin-2-phosphate reaction mixtures contain buffers, there is good agreement between its rate constants and those of lincomycin-2-phosphate at a given temperature and pH.

[1] TRIS

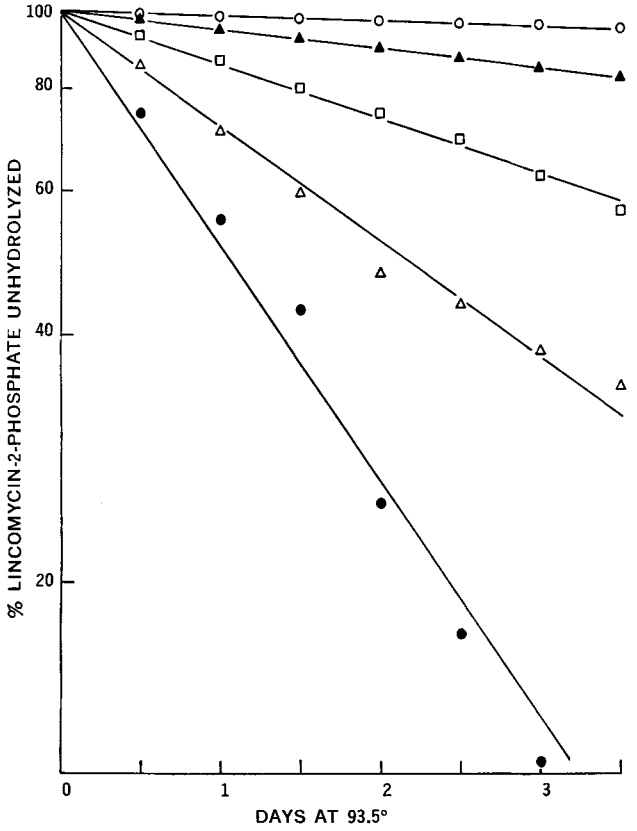

Figure 1—*Hydrolysis of lincomycin-2-phosphate at 93.5°. Key:* ○, *pH 8.4;* ▲, *pH 8.0;* □, *pH 7.5;* △, *pH 7.0;* ●, *pH 6.2.*

The pH-rate relationship for lincomycin-2-phosphate is shown in Fig. 2 for 59.4, 69.6, and 79.6° and in Fig. 3 for 90°. Above pH 5 the dependency of rate on pH is quite similar to that of monoalkyl phosphates such as methyl phosphate (9) and carbohydrate monophosphates such as α-D-glucose-1-phosphate (10). For monoalkyl phosphates and carbohydrate monophosphates the decrease in rate with increasing pH in this pH range is attributed to the decreasing concentration of the monoanionic species, the dianionic species being much less reactive. Since the pKa values of the phosphate moiety of lincomycin-2-phosphate are roughly 2 and 6 (4), the monoanionic species is present at maximum concentration at about pH 4, and the decrease in rate above pH 5 in Fig. 3 is probably due to decreasing concentration of monoanion.

Below pH 5 the rate of lincomycin-2-phosphate hydrolysis in Fig. 3 appears to be constant to about pH 1. In this pH range the two predominant species are the monoanion and the undissociated form of the phosphate moiety. The monoanion actually exists as a zwitterion and the undissociated form carries a net charge of +1 due to protonation of the amino group. (The pKa of lincomycin hydrochloride is 7.6.) The monoanion is present at maximum concentration at about pH 4 and decreases with decreasing pH, whereas the undissociated form increases in concentration as pH decreases in this range. The rate of hydrolysis in this pH range is the sum of the rates of hydrolysis of the monoanionic and undissociated form and this sum is a constant between pH 1 and 5. The pH profile of methyl phosphate shows a decrease in rate from pH 4 to pH 1 and this decrease is explained as corresponding to a decrease in the monoanion concentration, the undissociated form being relatively unreactive (9). On the other hand, the rate of glucose-1-phosphate hydrolysis increases rapidly between pH 5 and 1 and this increase is explained as due to the increase in concentration of undissociated glucose-1-phosphate (10) which is much more reactive than the corresponding form of methyl phosphate. Since the reactivities of the monoanionic species of lincomycin-2-phosphate (Table I), methyl phosphate (9), and glucose-1-phosphate (10) as well as hydroxyalkyl and alkyl monophosphates of similar structure (11) are of the same order, the plateau region of the lincomycin-2-phosphate pH profile between pH 1 and 5 indicates that the undissociated form of lincomycin-2-phosphate is more reactive than that of methyl phosphate but less reactive than the undissociated form of glucose-1-phosphate.

Table I—Apparent First-Order Rate Constants of Phosphate Ester Hydrolysis of Lincomycin-2-phosphate

pH	Temperature, °C.	$k \times 10^8$ sec.$^{-1}$
6.2	59.4	9.06
6.6	59.4	4.05
7.0	59.4	1.68
7.5	59.4	0.613
8.0	59.4	0.289
8.4	59.4	0.243
8.6	59.4	0.162
6.2	69.6	28.6
6.6	69.6	14.9
7.0	69.6	6.69
7.5	69.6	2.50
8.0	69.6	0.903
8.4	69.6	0.532
8.6	69.6	0.556
6.2	79.6	119
6.6	79.6	59.1
7.0	79.6	26.0
7.5	79.6	10.7
8.0	79.6	4.83
8.4	79.6	2.88
8.6	79.6	2.11
0.15[a]	90.0	5010
0.46[a]	90.0	2040
1.1[a]	90.0	1770
1.1[a]	90.0	765
2.0	90.0	735
2.1	90.0	875
3.0	90.0	749
3.1	90.0	719
4.0	90.0	755
4.2	90.0	725
5.0	90.0	668
5.1	90.0	708
6.6	90.0	309
7.4	90.0	66.4
8.0	90.0	12.2
8.5	90.0	8.89
9.6	90.0	4.23
6.2	93.5	941
6.6	93.5	356
7.0	93.5	184
7.5	93.5	61.5
8.0	93.5	23.8
8.4	93.5	14.3
8.6	93.5	11.3

[a] Calculated from pH = log f(HCl) where (HCl) is the experimental molarity and f is the mean activity coefficient for HCl at 90°, extrapolated from the literature (8).

There are at least two possible explanations for the sharp increase in rate of lincomycin-2-phosphate hydrolysis below pH 1 shown in Fig. 3. Both are based on the formation of new species which are more reactive than the undissociated form of lincomycin-2-phosphate. The first of these species may be the conjugated acid form of lincomycin-2-phosphate where conjugate acid is defined as the structure(s) resulting from protonation of the uncharged phosphate moiety. The other reactive species which may be formed at pH less than 1 is lincomycin-1-phosphate. Acid-catalyzed phosphoryl group

Table II—Apparent First-Order Rate Constants of Phosphate Ester Hydrolysis of Clindamycin-2-phosphate

pH	Buffer	Temperature, °C.	$k \times 10^8$ sec.$^{-1}$
7.50	0.2 *M* Tromethamine	59.5	0.757
7.50	0.2 *M* Tromethamine	70.0	3.61
7.50	0.2 *M* Tromethamine	80.0	14.6
5.95	0.2 *M* Ethylenediamine	90.4	278
6.40	0.2 *M* Ethylenediamine	90.4	185
6.80	0.2 *M* Tromethamine	90.4	72.7
7.30	0.2 *M* Ethylenediamine	90.4	69.5
7.40	0.2 *M* Tromethamine	90.4	41.3
7.50	0.2 *M* Tromethamine	90.4	46.8
7.75	0.2 *M* Tromethamine	90.4	24.4

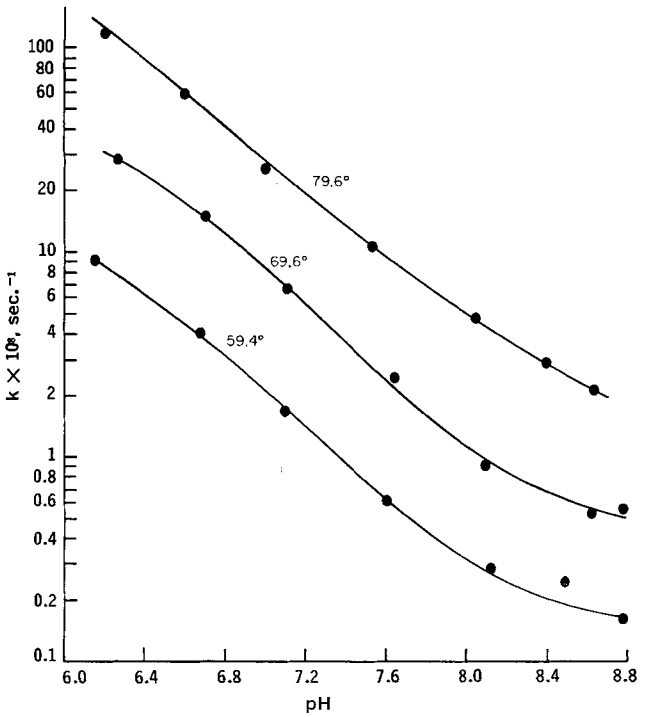

Figure 2—*pH-rate profile of phosphate ester hydrolysis of lincomycin-2-phosphate.*

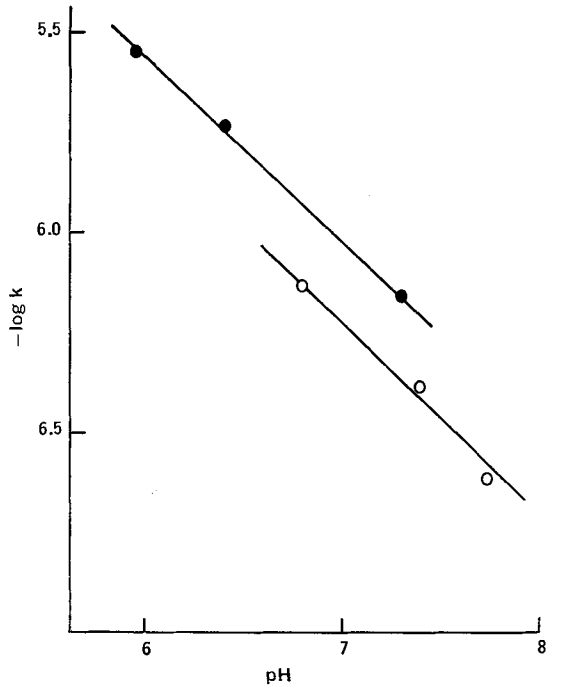

Figure 4—*Rate-pH profile of phosphate ester hydrolysis of clindamycin-2-phosphate at 90° and μ = 0.3. Key: ●, Tromethamine buffer; ○, ethylenediamine buffer.*

migration in monoesters containing suitably placed hydroxyl groups, such as lincomyin-2-phosphate, has been extensively reported (12, 13), and the new reactive species, lincomycin-1-phosphate, may be formed by phosphoryl group migration from C_2 to C_1. These migrations occur through formation of a five-membered cyclic intermediate (13). Glucose-1-phosphate is much more reactive than glucose-2-phosphate in this pH region (14) and by analogy lincomycin-1-phosphate would be expected to be more reactive than lincomycin-2-phosphate, thus causing the increase in observed hydrolysis rate below pH 1. A prerequisite to migration of the phosphoryl group from C_2 to C_1 in lincomycin-2-phosphate is cleavage of the thioglycoside bond to form the C_1 hydroxy compound. Thioglycoside hydrolysis has been reported as the major route of degradation of clindamycin in the pH range 0.4 to 4 (6). Extrapolation of these reported rate constants of thioglycoside hydrolysis to 90° reveals that the rate of thioglycoside hydrolysis is about five times greater than phosphate ester hydrolysis at pH 1 and falls to about two times greater at pH 4. Thus migration of the phosphoryl group from C_2 to C_1 is possible, and the increase in rate

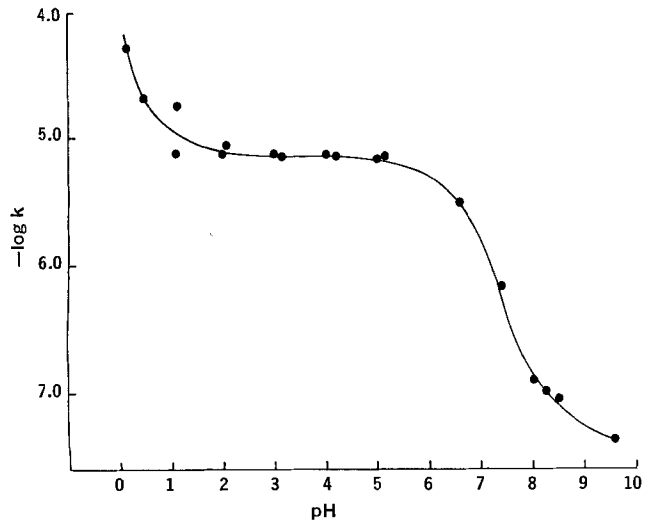

Figure 3—*pH-rate profile of lincomycin-2-phosphate hydrolysis at 90°.*

of lincomycin-2-phosphate hydrolysis below pH 1 may be due to the formation of the more reactive species, lincomycin-1-phosphate.

Migration of the C_2 phosphate group to the other adjacent hydroxyl at C_3 probably also occurs at pH < 1 (12), forming another species of the same order of reactivity as the 2-phosphate. However, it is unlikely that any phosphate migration occurs at higher pH since none was detected when lincomycin-2-phosphate was heated in 80% acetic acid (4), and alkaline migration does not occur in monoesters of this type (13).

The pH-rate relationship for phosphate ester hydrolysis of clindamycin-2-phosphate in ethylenediamine and tromethamine buffer in pH range 6–8 is shown in Fig. 4. Similar slopes of the two curves indicate that the rate of hydrolysis changes with pH to approximately the same extent in each buffer; however, nonoverlapping curves indicate that there is a buffer effect since at constant pH the rate is faster in ethylenediamine buffers. In both buffers, however, the pH-rate relationship is similar to that observed with lincomycin-2-phosphate between pH 6–8, *i.e.*, increasing hydrolysis rate as pH decreases.

The apparent first-order rate constants of degradation of clindamycin under conditions identical to clindamycin-2-phosphate are shown in Table III. According to a previous report (6), there are several products of clindamycin degradation in this pH range but the major degradative route is conversion to lincomycin. The influence of the phosphate ester moiety on the rate of lincomycin conversion is unknown when clindamycin alone is reacted; however, the relatively large distance between Positions 2 and 7 and the absence of conjugation minimize any substituent effects. The data in Table III indicate that the sum of the rate constants of lincomycin conversion and the other minor reactions is about 100 times greater than the rate of phosphate ester hydrolysis between pH 6 and 7.85. Thus, the phosphate ester moiety of clindamycin-2-phosphate is not the least stable portion of the molecule in this pH range. Extrapolation of rates of

Table III—Apparent First-order Rate Constants of Clindamycin Degradation at 90°C.

pH	Buffer	$k \times 10^6$ sec.$^{-1}$
6.05	0.2 *M* Ethylenediamine	1.05
6.90	0.2 *M* Tromethamine	2.58
7.40	0.2 *M* Tromethamine	4.80
7.85	0.1 *M* Tromethamine	6.32

Table IV—Activation Energy of Phosphate Ester Hydrolysis of Lincomycin-2-phosphate and Clindamycin-2-phosphate

Compound	pH	E_A, kcal./mole
Lincomycin-2-phosphate	6.20	33.3
Lincomycin-2-phosphate	6.65	32.1
Lincomycin-2-phosphate	7.05	33.5
Lincomycin-2-phosphate	7.55	33.1
Lincomycin-2-phosphate	8.05	32.2
Lincomycin-2-phosphate	8.40	30.1
Lincomycin-2-phosphate	8.65	30.4
Clindamycin-2-phosphate	7.50	32.9

phosphate ester hydrolysis and rates of degradation of other areas of the clindamycin-2-phosphate molecule to 25° shows that a liquid formulation adjusted to pH 7.4 would not meet stability criteria of less than 10% degradation after 2 years. However, these criteria are satisfied in the pH range 3.5 to about 6.5. Preliminary studies of lincomycin degradation alone indicate that the phosphate ester moiety is the least stable area of lincomycin-2-phosphate in the pH range 1–10. Calculations, using Tables I and IV data, show that lincomycin-2-phosphate meets the above stability criteria in the pH range 3.5–10.

Arrhenius plots of phosphate ester hydrolysis of lincomycin-2-phosphate at a variety of pH values and of clindamycin-2-phosphate at pH 7.5 in tromethamine buffer are shown in Fig. 5. Since clindamycin-2-phosphate was a parenteral product candidate, its activation energy was determined only at optimum physiological pH. Activation energies calculated from these curves are shown in Table IV. Good agreement between the activation energies of the two compounds was observed as expected since conversion of clindamycin to lincomycin is about 100 times faster than phosphate ester hydrolysis at this pH. Assuming that the monoanion is the predominant reactive species in the pH range reported in Table IV, the activation energies are in good agreement with values reported for phosphate esters of similar structure (11).

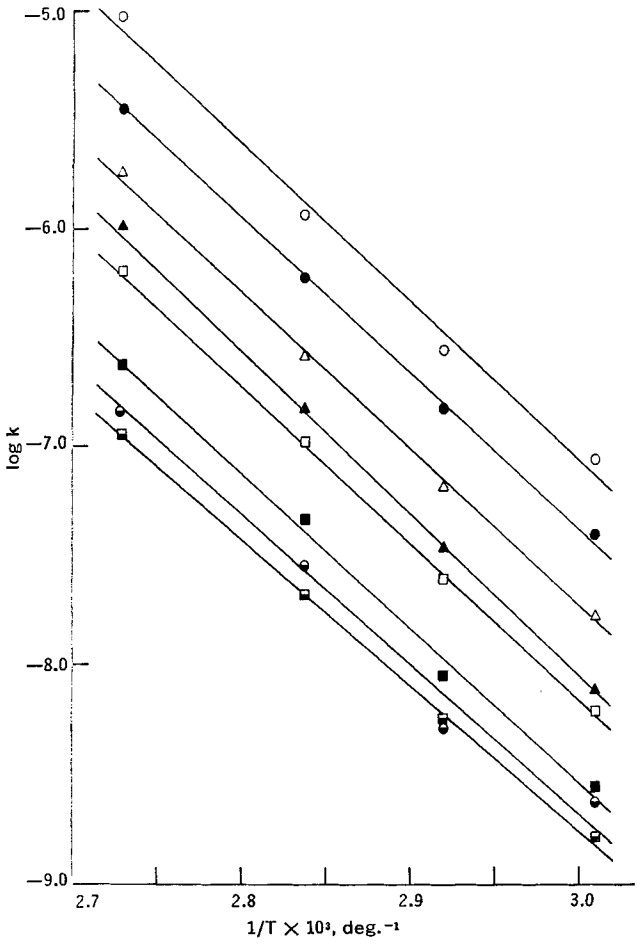

Figure 5—*Arrhenius plot of phosphate hydrolysis of lincomycin-2-phosphate and clindamycin-2-phosphate. Key:* ○, *lincomycin-2-phosphate, pH 6.20;* ●, *lincomycin-2-phosphate, pH 6.65;* △, *lincomycin-2-phosphate, pH 7.05;* ▲, *clindamycin-2-phosphate, pH 7.50;* □, *lincomycin-2-phosphate, pH 7.55;* ■, *lincomycin-2-phosphate, pH 8.05;* ◒, *lincomycin-2-phosphate, pH 8.4;* ◘, *lincomycin-2-phosphate, pH 8.65.*

Table V—Apparent First-order Rate Constants for Lincomycin-2-phosphate Hydrolysis in Pediatric Formulation

Temperature, °C.	$k \times 10^{10}$ sec.$^{-1}$ Predicted[a]	$k \times 10^{10}$ sec.$^{-1}$ Found
25	0.239	0.441
40	2.68	5.01
47	9.77	7.54
56	35.9	35.4

[a] Calculated from E_A = 32.1 kcal./mole and $k = 2.50 \times 10^{-8}$ sec.$^{-1}$ at 70°.

Accelerated stability studies of the type described in this study are useful in the prediction of product stability and determination of optimum formulation parameters. These studies in simple buffered solution provide baseline rate data for comparison with rates of degradation in actual formulations. Any influence of additives in a liquid formulation such as preservative, flavor, color, or solubilizing agent on the rate of drug degradation can be detected and corrected if necessary. Some early data on the hydrolysis of lincomycin-2-phosphate in a pediatric formulation containing sucrose, sorbitol, glycerin, preservatives, alcohol, saccharin, flavor, and color at a pH of 7.5 are shown in Table V along with rate constants predicted from 70° data in simple solution. Although hydrolysis had not occurred to greater than 3%, agreement between simple solution and formulation hydrolysis is reasonable and indicates that none of the formulation ingredients significantly influences lincomycin-2-phosphate hydrolysis.

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