ences for all comparisons of contractions per minute and its potential in intestinal studies (17). The effect upon interval between contractions could be explained on the basis of increased contractions per minute along with the observed decrease in amplitude of contractions. This would partially agree with earlier statements indicating strength, force, or possibly tone, but not amplitude of the contractions was increased (5).

Comparisons of activity between the high and low doses on the three parameters indicate that the high dose is more effective but with only the ileum comparisons of amplitude of contractions statistically significant and with no other segment specifically indicating a preferential effect.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 18, 1968 from the *Department of Pharmacology and Toxicology, School of Pharmacy, Northeast Louisiana State College, Monroe, LA 71201*

Accepted for publication June 5, 1969.

The authors wish to acknowledge Dr. L. T. Bennett and Dr. H. L. Dockery, Director, Academic Computer Center, for their assistance with the statistical analyses.

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Reactivity of the Hydroxyl Groups in Selected Derivatives of Lincomycin

W. E. HAMLIN

Abstract□ Reactivity of the hydroxyl groups in tris-2,3,4-*O*-trimethylsilyl lincomycin [$(TMS)_3$-L], lincomycin-2,7-diacetate·HCl (L-Ac_2) and 7-*O*-trityl-3,4-*O*-anisylidene lincomycin (TAL) as measured by the rate of acylation with valeric anhydride at room temperature was found to be in the order of 3-OH > 2-OH > 7-OH > 4-OH with estimated half-lives of 8, 32 and 160 min., and 23 hr., respectively. Gas chromatographic procedures were utilized to monitor the acylation of $(TMS)_3$-L and L-Ac_2. In the latter reaction silylation was necessary for measurement of unreacted starting material and the intermediate monovalerate. The acylation of TAL was monitored polarimetrically.

Keyphrases □ Lincomycin derivatives—hydroxyl groups, reactivity □ Acylation rate—lincomycin derivatives □ Polarimetry—reaction monitoring □ GLC—reaction monitoring

A consideration of a molecular model of lincomycin indicates that the steric and electronic environment of the hydroxyl groups at Positions 2, 3, 4, and 7 (Fig. 1) would differ and produce differences in reactivity. A knowledge of the relative reactivity of these hydroxyl groups can be useful in the synthesis of lincomycin derivatives. In order to obtain the desired information, the rates of acylation of tris-2,3,4-*O*-trimethylsilyl lincomycin, lincomycin-2,7-diacetate HCl, and 7-*O*-trityl-3,4-*O*-anisylidene lincomycin by valeric anhydride in pyridine were determined at room temperature. The acylating agent was used in such excess (20:1 mole ratio) that a pseudo-first-order reaction would apply.

EXPERIMENTAL

Acylation of Tris-O-2,3,4-trimethylsilyl Lincomycin [$(TMS)_3$-L]—This was an adaptation of a procedure for preparing lincomycin-7-acylates (1). To a solution of 1.428 g. of $(TMS)_3$-L (0.0025 mole) in 10 ml. of dry pyridine was added 9.31 g. (0.05 mole) of valeric anhydride (VA) at time zero. The solution was thoroughly mixed after adjusting to 25 ml. with dry pyridine and stored at room temperature. Sample aliquots (2 μl.) were removed by syringe and injected into the gas chromatograph at designated time intervals.

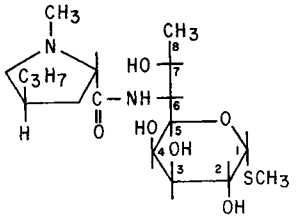

Figure 1—*Structure of lincomycin.*

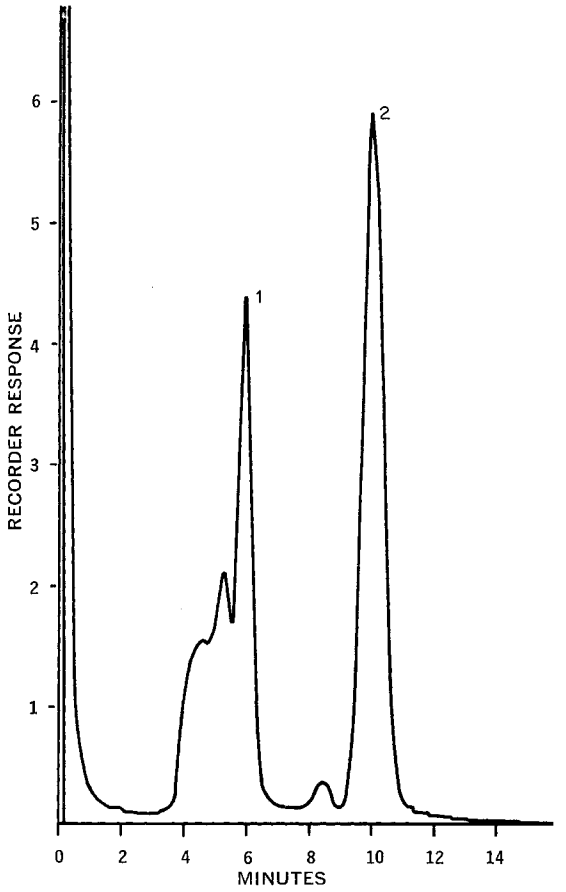

Figure 2—*Chromatogram of the acylation of (TMS)$_3$-L with valeric anhydride after 140 min at room temperature. Peak 1: (TMS)$_3$-L; Peak 2: (TMS)$_3$-L-V.*

A 0.63-cm. o.d. × 1.22-m. (0.25-in. o.d. × 4-ft.) stainless steel column packed with 3% SE-30 on 60/80-mesh Diataport S (Hewlett-Packard) was installed in a gas chromatograph (F & M model 810) (FID). The flow of helium carrier gas was maintained at 60 ml./min. The column oven was operated isothermally at 275° with the injector and detector temperatures at 300 and 335°, respectively. The relative amount of acylated product present at any given time was determined by measuring the area under the corresponding gas chromatographic peak by the height times the width at half-height method (see Table I).

Acylation of Lincomycin-2,7-diacetate.HCl (L-Ac$_2$)—To a solution of 1.225 g. (0.0025 mole) of L-Ac$_2$ in 10 ml. of dry pyridine was added 9.31 g. (0.05 mole) of VA at time zero. The solution was quickly adjusted to 25 ml. with dry pyridine, thoroughly mixed, and let stand at room temperature. At designated time intervals the acylation reaction in 100-μl. portions of the reaction mixture was quenched by adding with vigorous agitation to 1 ml. of a silylation reagent. After standing at room temperature for at least 15 min.,[1] a 2-μl. sample was injected into the gas chromatograph using the same parameters as described previously. The silylation reagent was prepared by dissolving 20 ml. of hexamethyldisilazane and 5 ml. of trimethylchlorosilane in sufficient dry pyridine to make 100 ml. of solution. One-milliliter portions of this reagent were pipeted into a number of 2-ml., glass-stoppered volumetric test tubes prior to the start of the acylation study. The areas (A_t) under the appropriate gas chromatographic peaks were measured as previously described. The area A_0 corresponding to the initial concentration of L-Ac$_2$ was determined by treating a blank solution containing no acylating agent. The area A_∞ corresponding to complete formation of L-Ac$_2$-V$_2$ was determined by heating the reaction mixture at 54° for 6 hr. after it had been at room temperature for more than 4 days.

[1] A preliminary test indicated that silylation was practically completed in about 1 min. A similarly rapid rate for the silylation of sugars has been reported by Sweeley *et al.* (2).

Table I—Reactivity of the Hydroxyl Groups in (TMS)$_3$-L, L-Ac$_2$, and TAL as Determined by Acylation with Valeric Anhydride

Lincomycin Derivative	Position of Hydroxyl Group Acylated	Estimated $T_{0.5}$ for Acylation with VA
(TMS)$_3$-L	7	160 min.
L-Ac$_2$	3	8 min.
	4	23 hr.
TAL	2	32 min.

Acylation of 7-O-Trityl-3,4-*O*-anisylidene Lincomycin (TAL) with with Acetic, Valeric, and Hexanoic Anhydrides—The acylation of TAL was an adaptation of a procedure for preparing lincomycin-2-acylates (3). The recording polarimeter (Bendix) with a 1-cm. fixed cell was used to follow the course of the reaction. A blank solution of 767 mg. of TAL in 10 ml. of dry pyridine was used to adjust the polarimeter to read zero on the chart for the start of the reaction. The range controls were adjusted to allow complete reaction to be recorded within the span of the chart. This was based on a preliminary trial run which had indicated the range of the change in optical rotation. In practice it was not necessary to measure the observed rotation which is proportional to the amount of ester formed as a function of time since the observed chart response is similarly related.

Procedure—To a solution of 767 mg. (0.001 mole) of TAL in 3 ml. of dry pyridine was added 0.02 mole of the anhydride with vigorous agitation (time zero on the recorder chart). The solution was adjusted to a 10-ml. volume with dry pyridine and let stand at room temperature. The 1-cm. cell was rapidly rinsed, filled with solution, and then placed in the polarimeter. From this time the course of the esterification reaction was continually plotted as a function of time. The value on the recorder when no further increase was recorded (R_∞) is proportional to 100% formation of the ester. Values at intermediate time intervals (R_t) were read from the chart.

RESULTS AND DISCUSSION

Acylation of (TMS)$_3$-L—A chromatogram of the reaction after 140 min. at room temperature (Fig. 2) was typical of those obtained.

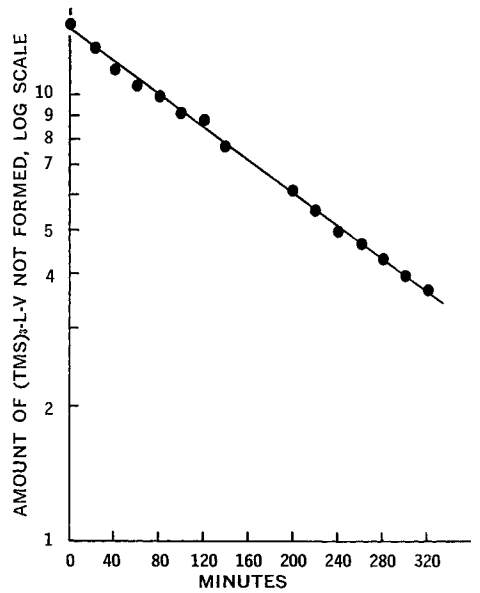

Figure 3—*The half-life of the acylation of (TMS)$_3$-L with VA at room temperature to form (TMS)$_3$-L-V. The amount of (TMS)$_3$-L-V not formed is measured as the area ($A_\infty - A_t$) under Peak 2 (Fig. 2). $t_{1/2} \sim 160$ min.*

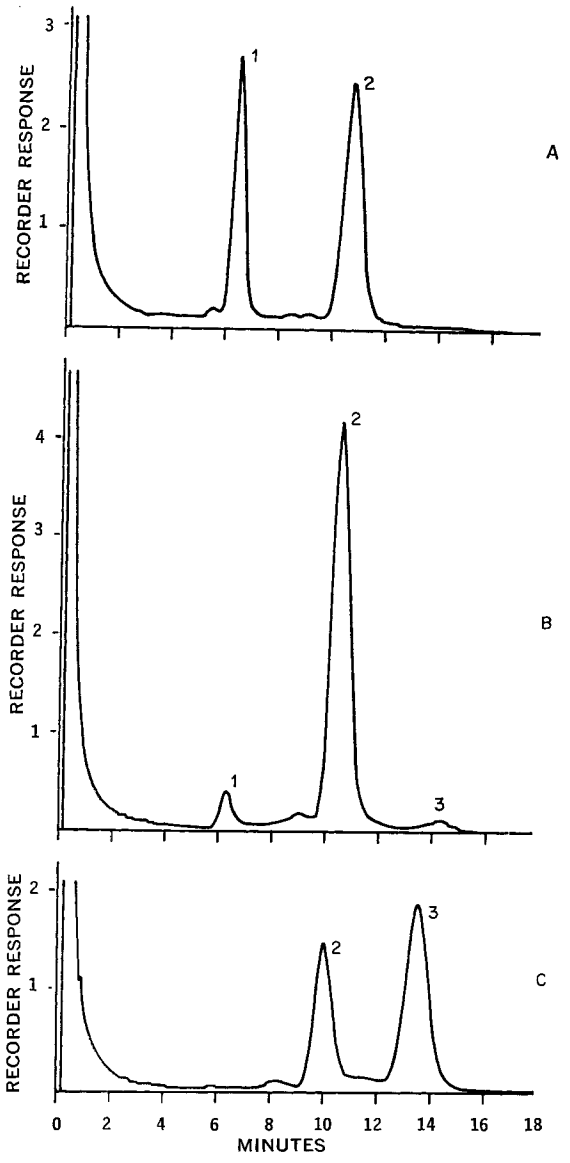

Figure 4—*Chromatograms of the silanized components of the acylation of L-Ac$_2$ with VA at room temperature after (A) 10 min.; (B) 40 min.; and (C) 35 hr. Peak 1 = L-Ac$_2$; Peak 2 = L-Ac$_2$-V; Peak 3 = L-Ac$_2$-V$_2$.*

Peak 1, with a retention time of 6.0 min., was due to unreacted (TMS)$_3$-L left in the solution. This peak was not considered suitable for quantitative use because of apparent decomposition as evidenced by unresolved components with slightly less retention times. Peak 2, with a retention time of 9.8 min., was due to the reaction product tris-2,3,4-*O*-trimethylsilyl lincomycin-7-valerate [(TMS)$_3$-L-V][2] and appeared suitable for quantitative treatment. The area A_t under Peak 2 was measured as a function of time. At completion of the reaction the area (A_∞) was found to be 14.5 cm.^{2}. A plot of log (A_∞-A_t), proportional to the amount of product not formed *versus* time in minutes (Fig. 3) was apparently first order by visual fit of the data. From this line the half-life of the acylation with valeric anhydride at the 7-position of (TMS)$_3$-L was found to be about 160 min.

Acylation of L-Ac$_2$—The unique feature of the experimental procedure was the silylation step which made possible the rapid and effective quenching of the reaction at designated time intervals while forming derivatives necessary for the gas chromatographic separation of L-Ac$_2$ and the monovalerate (L-Ac$_2$-V). Typical chromatograms of the acylation of L-Ac$_2$ with VA at room temperature shown in Fig. 4 indicated the rapid decrease in Peak 1 (L-Ac$_2$) with the corresponding increase in Peak 2 (L-Ac$_2$-V). The formation of the monoacylated product reached a maximum after about 40 min. If one had wanted to isolate this product with an optimum yield this would have been the time to quench the reaction. The formation of the di-valerate, Peak 3 (L-Ac$_2$-V$_2$), was quite slow as the mono-valerate, Peak 2, was consumed.

[2] This was proven by comparison to the gas chromatogram of authentic lincomycin-7-valerate as the tris-*O*-trimethylsilyl ether derivative under similar operating conditions.

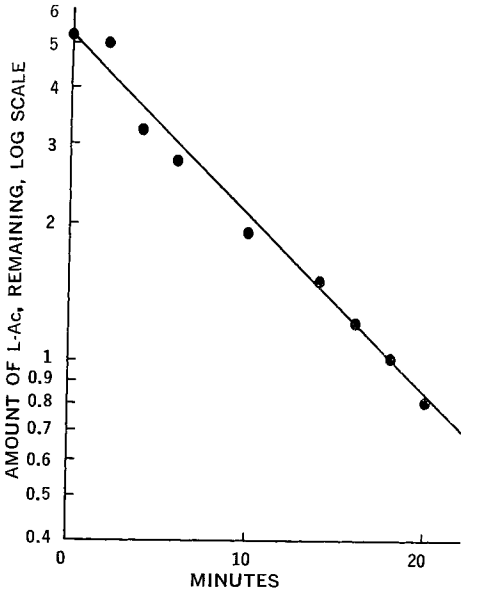

Figure 5—*The half-life of the acylation of L-Ac$_2$ with VA at RT to form L-Ac$_2$-V. The amount of L-Ac$_2$ remaining is measured as the area (A_t) under Peak 1 (Fig. 4). $t_{1/2} \sim 8$ min.*

The data indicated that two pseudo-first-order reactions were involved in the acylation of L-Ac$_2$ according to the kinetic model:

$$\text{L-Ac}_2 \xrightarrow{k_1} \text{L-Ac}_2\text{-V} \xrightarrow{k_2} \text{L-Ac}_2\text{-V}_2$$

This appears to be a reasonable assumption but has not been proven. A semilog plot of the amount of L-Ac$_2$ remaining, measured as the area (A_t) in cm.^{2}, under Peak 1 (Fig. 4) *versus* time in minutes (Fig. 5) was linear by visual fit of the data during the first 20 min. of the reaction. From this plot the half-life for the mono-valerated product, L-Ac$_2$-V, was estimated at 8 min.

Positive proof of whether mono-acylation occurred at the 3- or 4- position in L-Ac$_2$ was not obtained. However, there is evidence to suggest that the 3-hydroxyl group should be considerably more reactive to acylation than the 4-hydroxyl group. Such workers as Eliel and Lukach (4) and Buck *et al.* (5) have shown that the conformation of various cyclohexanols affects their reactivity to acylation with acid anhydrides. The hydroxyl group in an equatorial position is found to be more reactive than one in an axial position. The sugar moiety in the lincomycin molecule is somewhat analogous to

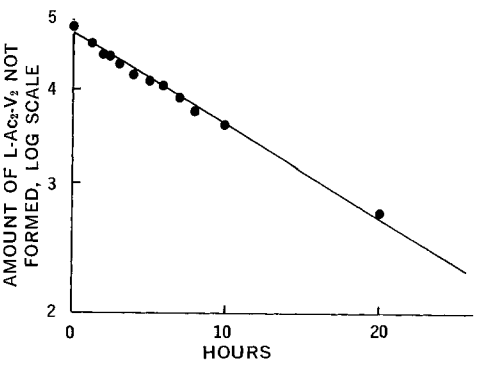

Figure 6—*The half-life of the acylation of L-Ac$_2$-V with VA at RT to form L-Ac$_2$-V$_2$. The amount of L-Ac$_2$-V$_2$ not formed is measured as the area ($A_\infty - A_t$) under Peak 3 (Fig. 4). $t_{1/2} \sim 23$ hr.*

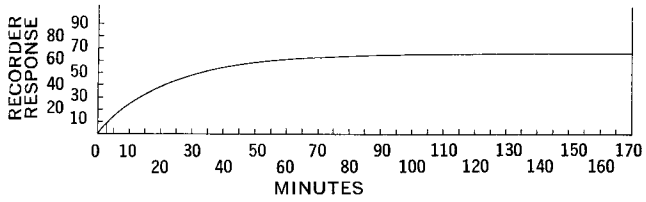

Figure 7—*The acylation of TAL with acetic anhydride at room temperature monitored continuously as a function of time by the recording polarimeter (Bendix).*

cyclohexanol since the hydroxyl at the 3-position is in the more reactive equatorial conformation as compared to the 4-hydroxyl which is axial (6).

As expected from the above discussion, the acylation of the 4-hydroxyl was quite slow as seen in Fig. 6 where log (L-Ac_2-V_2 not formed) measured as the area under Peak 3 (Fig. 4) was plotted as a function of time in hours. An apparent first-order reaction appeared to exist for about the first 20 to 30 hr. at room temperature. The half-life for this reaction was estimated at about 23 hr. Alternatively, a semilogarithmic plot of the amount of L-Ac_2-V remaining, measured as the area under Peak 2, Fig. 4, *versus* time (not shown) was linear from about 5 to 50 hr. The half-life estimated from these data was 26 hr.—essentially agreeing within experimental error with the first estimate of 23 hr. Obviously greater accuracy in the gas chromatographic assays could have been obtained by the use of an appropriate internal standard. However, the data obtained by the present method seem sufficiently accurate for the intended purpose, namely to determine the relative reactivity of the hydroxyl groups as an aid in the development of new synthetic approaches to derivative formation.

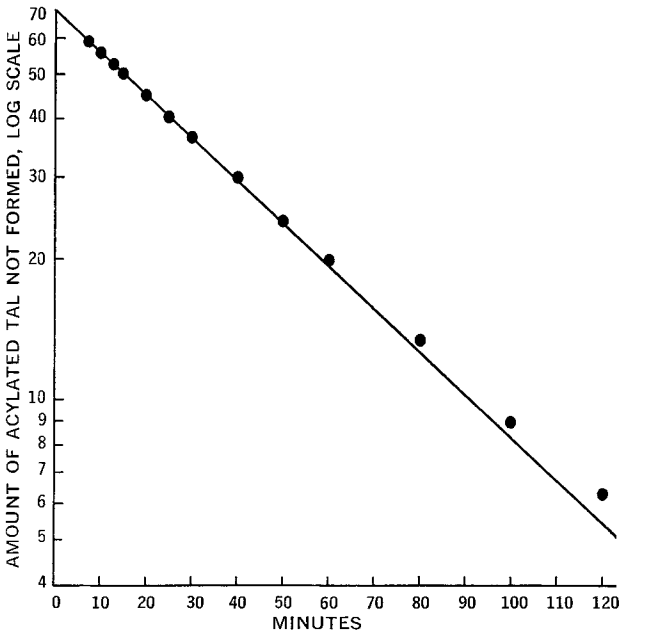

Figure 8—*The half-life of the acylation of TAL with valeric anhydride at room temperature. The amount of acylated TAL not formed was measured polarimetrically as the recorder response* ($R_\infty - R_t$). $t^1/_2 \sim 32$ *min.*

Acylation of TAL with Acetic, Valeric, and Hexanoic Anhydrides—The recorder response for the acylation of TAL with acetic anhydride (Fig. 7) was typical of the continuously monitored reactions by the polarimetric procedure. A similar tracing was obtained for the reaction of TAL with valeric and hexanoic anhydrides. Note that if R_∞ were the recorder response after complete reaction, and R_t were the recorder response at any designated intermediate time, then ($R_\infty - R_t$) is proportional to the amount of acylated product not formed. Thus, a semilog plot of the amount of acylated TAL not formed *versus* time in minutes (Fig. 8) indicated an apparent first-order reaction for the acylation of TAL with valeric anhydride having a half-life of about 32 min. In a like manner, the half-lives of the acylation reaction with acetic and hexanoic anhydrides were estimated to be 17 and 29 min., respectively.

The rate of acylation of TAL with acetic anhydride appeared to be about twice as fast as that with valeric or hexanoic anhydrides which were essentially equal within the limits of experimental error. This is consistent with theoretical considerations assuming that the steric effects of substituents in acylation with acidic anhydrides involve the same mechanism as in ester hydrolysis. Then, the equation

$$\log \frac{k}{k_0} = \rho^*\sigma^* + sE_s$$

provided by Taft (7) is applicable. A study of tables of σ^* and E_s values (7, 8) indicated that the rate of acylation should become progressively slower with increasing chain length of the alkyl substituent and that the rate will tend to "plateau" for the *n*-butyl or *n*-amyl substituents.

In summary, the reactivity of the free hydroxyl groups in $(TMS)_3$-L, L-Ac_2, and TAL as measured by the rate of acylation with valeric anhydride (Table I) is in the order of 3-OH > 2-OH > 7-OH > 4-OH. The order of reactivity in these derivatives should not be extrapolated to lincomycin itself without some reservations because the type of derivatization of hydroxyl groups at adjacent positions may have considerable effect on the reactivity of a particular hydroxyl group. However, this order of reactivity is a reasonable assumption at this time.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 2, 1969 from the *Pharmacy Research Unit, Pharmaceutical Research and Development Division, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication June 25, 1969.

Presented to the Basic Pharmaceutics Section APhA Academy of Pharmaceutical Sciences, Montreal meeting May 1969.

The technical assistance of Mr. J. W. Woltersom during the course of this investigation was greatly appreciated.