Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ACIDIC AND ALKALINE ISOMERIZATION AND HYDROLYSIS OF LINCOMYCIN MONOESTERS

T. O. OESTERLING

Pharmacy Research Unit, The Upjohn Company, Kalamazoo, Michigan 49001 (U. S. A.)

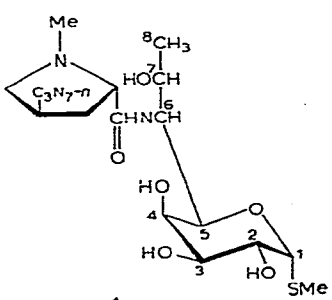
(Received March, 7th, 1970; in revised form, May 23rd, 1970)

ABSTRACT

In alkaline media the 2-, 3-, and 4-monohexanoates of lincomycin rapidly isomerize, each entering into facile equilibrium with the other two, with accompanying hydrolysis of each species to lincomycin. In acid media, lincomycin 3-hexanoate and 4-hexanoate isomerize to form a mixture of 3- and 4-hexanoates and a trace of the 2-hexanoate, with accompanying hydrolysis to lincomycin. Lincomycin 2-hexanoate, however, does not isomerize under these conditions and undergoes straightforward hydrolysis. Lincomycin 7-hexanoate undergoes straightforward hydrolysis at low or high pH to form lincomycin and hexanoic acid. Based on the time required for one-half of the initial compound to disappear, the relative alkaline stability of the four monoesters is $3<4<2\ll$7-hexanoate. Based on the time required for one-half of the total possible lincomycin to appear, the relative order of alkaline stability is $2<3=4\ll$7-hexanoate. In acidic media, based on the time required for one-half of the initial compound to disappear or the time required for one-half of the total possible lincomycin to appear, the relative order of stability is the same, $2=3=7<4$-hexanoate.

INTRODUCTION

The synthesis and biological activities of the four possible monoesters of lincomycin (**1**) have been reported by Morozowich *et al.*[1–4]. These compounds were prepared as part of a program whose object was to improve the pharmaceutical and biological properties of parent drug through derivatives that are reversible *in*



1

vivo. This report describes the relative stabilities of the monohexanoates of lincomycin in acidic and alkaline media and outlines the mechanism by which deacylation occurs.

EXPERIMENTAL

General methods. — Lincomycin, lincomycin 2-hexanoate, lincomycin 3-hexanoate, lincomycin 4-hexanoate, and lincomycin 7-hexanoate containing less than 2% of impurities were supplied by the research laboratories of The Upjohn Company. All other chemicals were reagent grade. I.r. spectra of Nujol mulls of the samples were recorded with a Perkin–Elmer Model 137 spectrophotometer. G.l.c. was performed on an F & M Model 402 instrument, and mass spectra were recorded with an LKB 9000 gas chromatograph–mass spectrometer. Samples for t.l.c. were spotted on plates of Silica Gel G, developed with 60:20:20:9:10, hexane–ether–2-pentanone–methanol–concentrated ammonium hydroxide, and visualized by heat after spraying with 30% aqueous ammonium sulfate.

Kinetic studies. — Alkaline reaction-mixtures were prepared by dissolving 50 mg of the lincomycin monoester in 5 ml of methanol, mixing with 5 ml of 0.2M carbonate buffer (pH 9.5), and placing the mixtures in a constant-temperature bath at 25°. At appropriate times 1.0 ml aliquots were withdrawn, mixed with 100 μl of glacial acetic acid, and freeze-dried. The resulting cakes were assayed by the procedure described below.

Acidic reaction mixtures were prepared by dissolving 50 mg of lincomycin monoester in 5 ml of methanol, mixing with 5 ml of M hydrochloric acid, and maintaining the mixtures at 37°. At appropriate times 1.0 ml aliquots were withdrawn, mixed with 100 μl of 7M ammonium hydroxide and freeze-dried. The resulting cakes were assayed by the procedure described below.

To each freeze-dried sample was added 500 μl of a 9:1 solution of *N,O*-bis-(trimethylsilyl)acetamide–chlorotrimethylsilane containing 10.0 mg/ml of cholesteryl monochloroacetate. After mixing for 5 min, 1 μl of each sample was injected into the gas chromatograph adjusted to the following conditions. Glass columns 120 cm × 3 mm (inside diameter) were packed with 1% OV-1 on Gas Chrom Q 60–80 mesh and preconditioned by heating at 300° for at least 6 h with a low helium flow rate and then for one hour under no-flow conditions. The carrier gas helium flowed at 50 ml/min and the flow rates of air and hydrogen were adjusted to yield maximal response. The column temperature was 245°, flash-heater temperature 245°, and flame-ionization detector 270°.

The amount of each species present in each sample was determined by comparing ratios of peak heights for each species with the internal standard to the peak-height ratios of standard curves prepared from known amounts of each species.

RESULTS

A typical gas–liquid chromatogram obtained by processing an aliquot from the alkaline degradation of lincomycin 2-hexanoate is shown in Fig. 1. Data similar

to those of Fig. 1 were obtained from degradation of the 2-, 3-, or 4-hexanoates in alkaline media and from degradation of the 3- and 4-hexanoates in acid media, the only difference being the rates of increase and decrease of the various peaks. Chromatograms from degradation of the 7-hexanoate at high pH and of the 2- and 7-hexanoates at low pH showed only two peaks, representing starting material (ester), and product (lincomycin). Hexanoic acid formed in the reaction mixtures was not observed by g.l.c. analysis since it was either removed during the freeze-drying step or eluted at the solvent front.

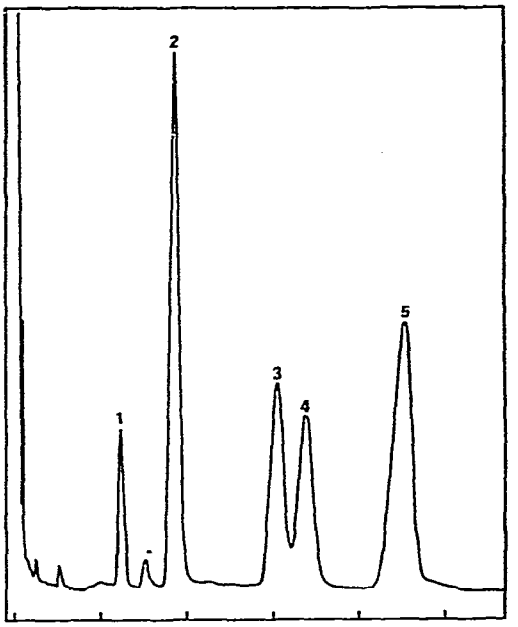


Fig. 1. Typical gas–liquid chromatogram obtained by processing an aliquot from the alkaline hydrolysis of lincomycin 2-hexanoate. Peak 1, $(TMS)_4$-lincomycin; peak 2, $(TMS)_3$-lincomycin 4-hexanoate; peak 3, $(TMS)_3$-lincomycin 3-hexanoate; peak 4, $(TMS)_3$-lincomycin 2-hexanoate; peak 5, cholesteryl monochloroacetate (internal standard).

Authentic samples of silylated lincomycin and 2-, 3-, and 4-hexanoates had the same retention times as the corresponding peaks in Fig. 1 when chromatographed under identical conditions. The small peaks preceding the $(TMS)_4$-lincomycin and $(TMS)_3$-lincomycin 4-hexanoate peaks are the trimethylsilyl (TMS) derivatives of the 4′-ethyl analogs. A small proportion of the 4′-ethyl analog was present as impurity in each of the lincomycin monohexanoates.

Mass spectra of peaks 2, 3, and 4 of Fig. 1 all showed an ion at *m/e* 720 that was taken as the molecular ion and corresponds to the molecular weight of a silylated lincomycin monohexanoate. The mass spectrum of peak 1 in Fig. 1 showed a molecular-ion signal at *m/e* 694, which corresponds to the molecular weight of $(TMS)_4$-lincomycin. T.l.c. of alkaline reaction-mixtures following reaction of the 2-, 3-, or 4-hexanoate showed three spots, all of which corresponded by t.l.c. with a mixture of

authentic lincomycin and its 2-, 3-, and 4-hexanoates (the 3- and 4-hexanoates have the same R_F value). No unidentified zones were observed. I.r. spectra of material collected by preparative g.l.c. representing peaks 2, 3, and 4 of Fig. 1 were nearly identical with each other and with authentic samples of the appropriate silylated monoesters. The i.r. spectrum of peak 1 in Fig. 1 was identical with that of $(TMS)_4$-lincomycin. Products in the reaction mixtures from lincomycin 7-hexanoate were identified by the same techniques described above.

The relative stabilities of the various lincomycin monoesters are shown in Tables I and II. Half-lives for starting materials were established by noting the time

TABLE I

STABILITIES OF LINCOMYCIN MONOESTERS IN 50% 0.2M CARBONATE BUFFER (pH 9.5)–50% METHANOL AT 25°.

Ester	$t_{\frac{1}{2}}$ *for initial compound (min)*	$t_{\frac{1}{2}}$ *for lincomycin formation (min)*
2-Hexanoate	4.5 ±1.5	26.0 ±1.0
3-Hexanoate	0.7 ±0.1	34.8 ±1.2
4-Hexanoate	2.5 ±0.5	34.3 ±3.1
7-Hexanoate	550 ±30	550 ±30

TABLE II

STABILITIES OF LINCOMYCIN MONOESTERS IN 50% M HCl–50% ETHANOL AT 37°.

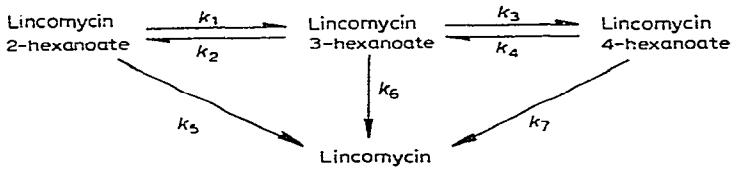
Ester	$t_{\frac{1}{2}}$ *for initial compound (h)*	$t_{\frac{1}{2}}$ *for lincomycin conversion (h)*
2-Hexanoate	17 ±5	17 ±5
3-Hexanoate	15 ±5	19 ±5
4-Hexanoate	75 ±10	90 ±10
7-Hexanoate	20 ±5	20 ±5

required for the peak-height ratio to decrease to one-half. Half-lives for conversion into lincomycin were obtained from plots of log(theoretical peak-height ratio of lincomycin at total conversion minus peak-height ratio at t) *vs.* time. In all cases complete conversion of starting material and intermediates into lincomycin was observed.

DISCUSSION

Acyl migration from one hydroxyl group to a proximate one in the same molecule has been reported many times. Monoacyl glycerides can rearrange under the influence of acid, base, or enzyme catalysis[5], and acyl groups attached to sugars containing free hydroxyl groups can migrate in dilute acid or alkali to occupy new positions[6,7,8]. In all cases, the migration is thought to occur through an ortho acid or ortho ester intermediate first postulated by Fischer[9].

For the lincomycin 2-, 3-, and 4-hexanoates, the following scheme describes the reaction pathway in alkaline media:



since each of these species was detected in the reaction mixture during the course of the reaction, regardless of starting material. Under acid conditions, however, it appears that k_1 and k_2 are very small compared with the other rate constants, since no 3- or 4-ester was found when the 2-hexanoate was used as starting material and only a trace of the 2-ester was found when the 3- or 4-hexanoate was used as starting material. Formation of isomeric monoesters of lincomycin most likely proceeds through cyclic intermediates such as those postulated for similar migrations[8,9].

Preliminary experiments with other monoacyl esters of lincomycin indicated they are degraded by the same route shown above for lincomycin monohexanoates. T.l.c. and g.l.c. of alkaline reaction-mixtures of the 2-butyrate, 2-valerate, and 2-palmitate of lincomycin indicated the presence of positional isomers during degradation, before the ultimate formation of the parent alcohol.

In alkaline reaction-mixtures the data showed that acyl migration from position 3 is favored to position 4 over position 2 and this preference is also evident in acidic reaction mixtures, where no migration between positions 2 and 3 was observed. This favored migration of acyl groups between positions 3 and 4 is undoubtedly due to the relative ease of formation of the 3,4-*cis*, rather than the more strained 2,3-*trans*, cyclic intermediate involved in the process[10]. Synthesis of acetals of lincomycin yields 3,4-acetals and not 2,3-acetals[11,12], and acyl migration between vicinal hydroxyl groups of furanose sugars occurs more readily when the substituents are cis[13].

Since migration as well as hydrolysis is involved in the degradation of lincomycin monoesters, the data were evaluated by two methods (*a*) the time required for one-half of the starting material to be degraded, and (*b*) the time required for one-half of the product to appear. This technique illustrates the influence of isomerization, if any, on the relative stabilities of the esters, and yields information such as the relative magnitudes of the rate constants for isomerization and hydrolysis. For example, at high pH (Table I) the half-lives for disappearance of the 2-, 3-, and 4-hexanoates are all much smaller than the half-lives for formation of product, which indicates that all isomerization processes are faster than hydrolysis in this case. On the other hand, considering the 3- and 4-hexanoates (Table II), the rates of isomerization and hydrolysis are not markedly different in acid media. A study, in which values of the rate constants k_1 through k_7 were determined by computer analysis of the data for the alkaline degradation of the 2-, 3-, and 4-hexanoates, is reported elsewhere[14].

The reactivity of the 7-hexanoate, in comparison with that of the monoesters of the pyranoside moiety, is markedly different in acid and base. At high pH the half-life for formation of lincomycin is about 100 times greater for hydrolysis of the 7-hexanoate, whereas in 0.5M methanolic hydrogen chloride the 7-ester reacts at approximately the same rate as the 2-, 3-, and 4-esters. One possible explanation for the difference in alkaline media may be the increased reactivity of the 2-, 3-, and 4-monoesters because of facilitation of hydrolysis by neighboring hydroxyl groups[15,16]. The report that steric and resonance effects are the same in acid- and base-catalyzed ester hydrolyses, whereas polar effects of substituents are markedly greater in alkaline media[17], supports this explanation. The relatively low reactivity of the 4-hexanoate at low pH is probably due to steric hindrance arising from the axial orientation of the C-4 substituent.

ACKNOWLEDGMENTS

The author thanks Dr. P. B. Bowman for obtaining and aiding in the interpretation of mass spectra, and Mr. G. R. Munting for excellent technical assistance in this work.

REFERENCES

1 W. MOROZOWICH, A. A. SINKULA, F. A. MCKELLAR, AND C. LEWIS, *J. Pharm. Sci.*, in press.
2 A. A. SINKULA, W. MOROZOWICH, C. LEWIS, AND F. A. MCKELLAR, *J. Pharm. Sci.*, 58 (1969) 2140.
3 W. MOROZOWICH, F. A. MCKELLAR, AND C. LEWIS, *J. Pharm. Sci.*, in press.
4 W. MOROZOWICH, F. A. MCKELLAR, AND C. LEWIS, *J. Pharm. Sci.*, in press.
5 D. J. HANAHAN, *Lipid Chemistry*, John Wiley and Sons, Inc., New York, 1960, p. 194.
6 W. PIGMAN, *The Carbohydrates*, Academic Press, Inc., New York, 1957, p. 147.
7 J. M. SUGIHARA, *Carbohyd. Chem.*, 8 (1953) 2.
8 E. PASCU, *Advan. Carbohyd. Chem.*1, (1945) 111.
9 E. FISCHER, *Ber.*, 53 (1920) 1621
10 S. J. ANGYAL AND C. G. MCDONALD, *J. Chem. Soc.*, (1952) 686; A. N. DE BELDER, *Advan. Carbohyd. Chem.*, 20 (1965) 219.
11 H. HOEKSEMA, B. BANNISTER, R. D. BIRKENMEYER, F. KAGAN, F. A. MCKELLER, W. SCHROEDER, G. SLOMP, B. J. MAGERLEIN, AND R. R. HERR, *J. Amer. Chem. Soc.*, 86 (1964) 4223.
12 M. J. TARASZKA AND W. MOROZOWICH, *J. Org. Chem.*, 33 (1968) 2349.
13 S. TEJIMA AND H. G. FLETCHER, JR., *J. Org. Chem.*, 28 (1963) 2999.
14 T. O. OESTERLING AND C. M. METZLER, *J. Pharm. Sci.*, in press.
15 H. B. HENBEST AND B. J. LOVELL, *J. Chem. Soc.*, (1957) 1965.
16 T. C. BRUICE AND T. H. FIFE, *J. Amer. Chem. Soc.*, 84 (1962) 1973.
17 R. W. TAFT, JR., *Steric Effects in Organic Chemistry*, M. S. NEWMAN, Ed., John Wiley and Sons, Inc., New York, 1956.