

## REFERENCES

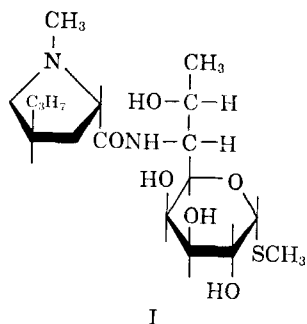
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## Acid Stability of Lincomycin

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Lincomycin degrades slowly in 0.1 N HCl at 70° (half-life, 39 hr.), and the decrease in optical rotation shows a direct correlation with microbiological assays. At 37°, lincomycin shows no degradation for at least 48 hr. in 0.1 N HCl. Under these conditions, penicillin V degrades with a half-life of 29 min.

THE ISOLATION, characterization, microbiological assay, and biological evaluation of the new Gram-positive specific antibiotic, lincomycin,<sup>1</sup> have been reported recently together with absorption and excretion studies in man and animals (1-9). Structure I has been assigned to lincomycin (10). Cryst-



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talline lincomycin hydrochloride was found to be stable for at least 6 months at 70° (2). To predict the stability of this antibiotic in the stomach following oral administration, studies have been conducted in acid solution at 37° and 70°. A comparative examination of the acid stability of penicillin V at 37° was also made.

## EXPERIMENTAL

**Lincomycin Degradation at 70°.**—A 0.4% solution of lincomycin hydrochloride in 0.1 N HCl was incubated at 70°. At intervals over a period of 389 hr., 10-ml. aliquots were removed for potentiometric titrations. At the same time, 5-ml. aliquots were removed and frozen for subsequent optical rotation and spectral measurements and 100- $\mu$ l. aliquots were diluted to 25 ml. with 0.1 M phosphate buffer, pH 7.0, and frozen for bioassay by agar diffusion versus *Sarcina lutea* (3).

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<sup>1</sup>Lincomycin hydrochloride. Marketed as Lincocin by The Upjohn Co., Kalamazoo, Mich.

Titration were run immediately after sampling. Following addition of 9 ml. of 0.1 N NaOH to each sample, titrations were completed potentiometrically with 1 N NaOH. Twenty milliliters of ethanol then was added to each sample and the solutions back-titrated with 1 N HCl. Consumption of acid or base and apparent pKa's were calculated for each sample (Table I).

**Lincomycin Degradation at 37°.**—A 0.4% solution of lincomycin hydrochloride in 0.1 N HCl was incubated at 37°. At intervals over a period of 48 hr., 10-ml. aliquots were removed. From each aliquot, a 100- $\mu$ l. portion was removed and diluted to 25 ml. with 0.1 M phosphate buffer, pH 7.0. Three-milliliter aliquots of the resulting buffered solutions were diluted further with equal volumes of the phosphate buffer. All the buffered solutions were frozen for subsequent microbiological assay. The remainder of each sample aliquot was frozen for optical rotation determinations.

**Penicillin V Degradation at 37°.**—A 10-mg. sample of penicillin V was dissolved in 45 ml. of water in a 50-ml. volumetric flask and brought to 37° in a water bath. A 5-ml. portion of 1 N HCl was added, the solution was mixed, and a 2-ml. aliquot was removed. The aliquot was quickly added to 8 ml. of 0.1 M phosphate buffer, pH 7.0,

TABLE I.—POTENTIOMETRIC TITRATION STUDIES OF LINCOMYCIN AS A FUNCTION OF DEGRADATION TIME AT 70°C. IN 0.1 N HCl

Time, hr.	Meq. of Amine/ml.		pKa'	
	H <sub>2</sub> O	51% EtOH	H <sub>2</sub> O	51% EtOH
0	0.0095	0.0092	7.92	7.00
1.8	0.0101	0.0092	8.00	...
5.5	0.0097	0.0093	7.94	...
22.5	0.0104	0.0093	7.95	...
46.5	0.0096	0.0094	7.92	6.84
77.5	0.0096	0.0095	7.92	...
150	0.0101	0.0093	7.95	...
249	0.0094	0.0092	7.87	...
389	0.0094	0.0093	7.80	7.12
Mean	0.0098	0.0093	7.92	6.99
S.D.	0.0004	0.0001	0.06	0.14

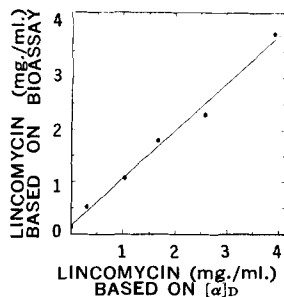


Fig. 1.—Correlation of lincomycin bioassay with optical rotation assay on material degraded at 70°C. in 0.1 N HCl.

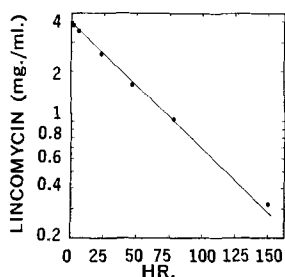


Fig. 2.—Pseudo first-order degradation of lincomycin at 70°C. in 0.1 N HCl (based on optical rotation).

TABLE II.—LINCOMYCIN STABILITY AT 37°C. IN 0.1 N HCl

Time, min.	Lincomycin Concn., mg./ml.		
	Optical Rotation <sup>a</sup>	I <sup>b</sup>	II <sup>c</sup>
0	3.90	5.00	3.05
30	3.99	> 5.00 <sup>d</sup>	3.75
60	3.99	4.38	7.60
120	4.10	5.00	8.00
180	4.02	> 5.00 <sup>d</sup>	4.20
280	4.00	> 5.00 <sup>d</sup>	4.45
360	4.01	2.75	4.00
1287	4.00	3.55	3.75
1525	4.01	> 5.00 <sup>d</sup>	5.00
1750	3.99	3.55	4.20
2710	4.01	3.80	5.00
2830	3.99	2.63	5.00
Mean	4.00	...	4.43
S.D.	0.04	...	1.37

<sup>a</sup> Based on  $[\alpha]_D = 138.4^\circ$ . <sup>b</sup> Dilution with phosphate buffer (1:250) employed for assay. <sup>c</sup> Dilution with phosphate buffer (1:500) employed for assay. <sup>d</sup> Values not included in mean determination.

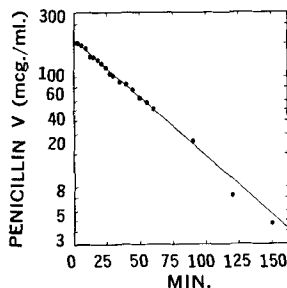


Fig. 3.—Pseudo first-order degradation of penicillin V at 37°C. in 0.1 N HCl (based on bioassay).

in an ice-water bath and, after mixing, frozen for bioassay by agar diffusion versus *Staphylococcus aureus* (ATCC 6538P). Subsequent aliquots of the reaction mixture were taken at appropriate time intervals and treated similarly.

## RESULTS AND DISCUSSION

Degradation of lincomycin in 0.1 N HCl at 70°C was followed by optical rotation measurements, potentiometric titrations, ultraviolet spectroscopy, and microbiological assays. Decreases in bioactivity and specific rotation occurred, and antibiotic content calculated on the basis of  $[\alpha]_D$  showed a direct correlation with the microbiological assay results (Fig. 1). A linear regression of bioassay ( $y$ , milligrams per milliliter) versus optical rotation assay ( $x$ , milligrams per milliliter) gave the equation  $y = 0.914x + 0.159$ , with 95% confidence intervals of 0.797 to 1.031 and  $-0.084$  to 0.402 for the slope and intercept, respectively. Figure 2 (based on  $[\alpha]_D$  values) shows that degradation of lincomycin was pseudo first order with a half-life of 39 hr. The acid degradation involves cleavage of the thioether with production of methanethiol (10).

Results in Table I show that no consumption or production of acid was observed, and the pKa' of the titratable group in the antibiotic remained unchanged during degradation at 70°C over a period of 389 hr. No useful ultraviolet spectral changes occurred.

Examination of rotation and bioassay values for lincomycin in 0.1 N HCl at 37°C gave the results shown in Table II. Total stability under these conditions for at least 48 hr. is indicated. It may be concluded that instability of lincomycin in the stomach or under conditions to be encountered during formulation is not a problem.

To provide comparative data on the stability of a widely used antibiotic, the rate of degradation of penicillin V in 0.1 N HCl at 37°C was determined by microbiological assay. Results in Fig. 3 indicate pseudo first-order degradation with a half-life of 29 min. under these conditions, in agreement with the results of Gourevitch *et al.* (11). A half-life of 1.65 min. has been reported for erythromycin at pH 2.0 and 25°C (12).

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