Synthesis and Bioactivity of Lincomycin 2-Monoesters

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Abstract □ A homologous series of straight-chain lincomycin 2-monoesters, comprised of both acyl and carbonate esters, was synthesized. Good antibacterial activity was observed with esters of chain lengths from C_4 to C_{16}. Esters with chain lengths from C_{12} to C_{16} are tasteless and highly active *in vivo* in mice infected with *Staphylococcus aureus*. The carbonate esters show essentially the same taste and bioactivity as the acyl esters. The long-chain lincomycin 2-esters appear to have the desired properties required for formulation as a tasteless pediatric preparation.

Keyphrases □ Lincomycin 2-monoesters—synthesis, antibacterial activity, tasteless long-chain esters □ Antibacterial agents, potential—synthesis of lincomycin 2-monoesters □ Pediatric formulations, potential—synthesis, antibacterial activity of long-chain lincomycin 2-monoesters □ Tasteless lincomycin derivatives—synthesis, antibacterial activity of 2-monoesters

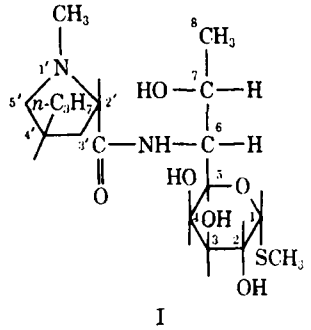
This work is part of a project designed to improve the pharmaceutical properties (*e.g.*, taste and solubility) and biological properties (*e.g.*, absorption and prolonged activity) of the antibiotic lincomycin (I) (1–3) by means of various *in vivo* reversible monoesters.

Molecular models show that each of the four hydroxyl groups in lincomycin is confronted with different steric and electronic environments. The *in vivo* rate of hydrolysis of the four possible positionally isomeric esters is probably determined by the ease of hydrolysis by esterase enzymes or by nucleophiles, such as hydroxide ion. Polyesters of lincomycin show poor antibacterial activity *in vivo*, probably because some esterified hydroxyl groups are more difficult to hydrolyze than others. In view of this possibility, monoesters at each of the four hydroxyl groups in I were synthesized in an attempt to find a hydroxyl that would provide biologically active *in vivo* reversible esters with the desired pharmaceutical or biological properties. Protective group routes were perfected for the synthesis of each of the four isomeric lincomycin monoesters. This report describes the synthesis and properties of lincomycin 2-monoesters, and future reports will deal with the 3- and 4-monoesters.

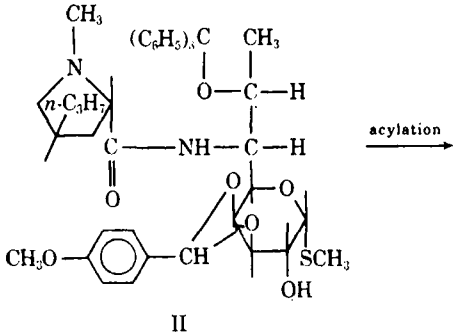
The primary objective of this work was to uncover a tasteless, bioactive lincomycin ester with low water solubility, which would permit formulation of a pharmaceutically elegant pediatric suspension. This objective was met with the long-chain lincomycin 2-monoesters.

RESULTS AND DISCUSSION

Chemistry—The lincomycin 2-monoesters were synthesized (Scheme I) by reaction of the key intermediate 7-*O*-trityl-3,4-*O*-anisylidene lincomycin (II) (4) with an acid chloride, an acid anhydride, or a chlorocarbonate in the presence of pyridine. The resulting 7-*O*-trityl-3,4-*O*-anisylidene lincomycin 2-esters (III) were treated with 80% aqueous acetic acid at 100° for 15–30 min. to remove the trityl and anisylidene protective groups. In most cases, the lincomycin 2-monoesters (IV) were maintained as the hydrochloride salts during workup and purification. The esters are un-



I



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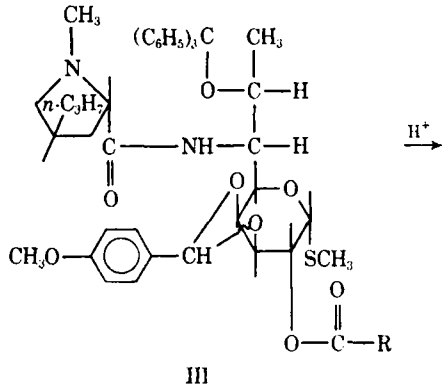

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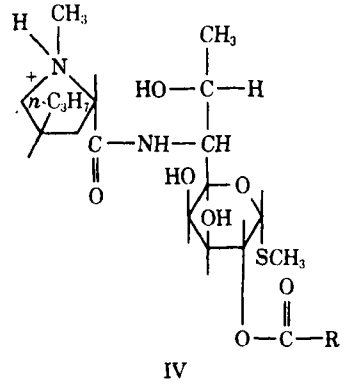

IV

Scheme I—$R = -(CH_2)_nCH_3$ or $-O(CH_2)_nCH_3$

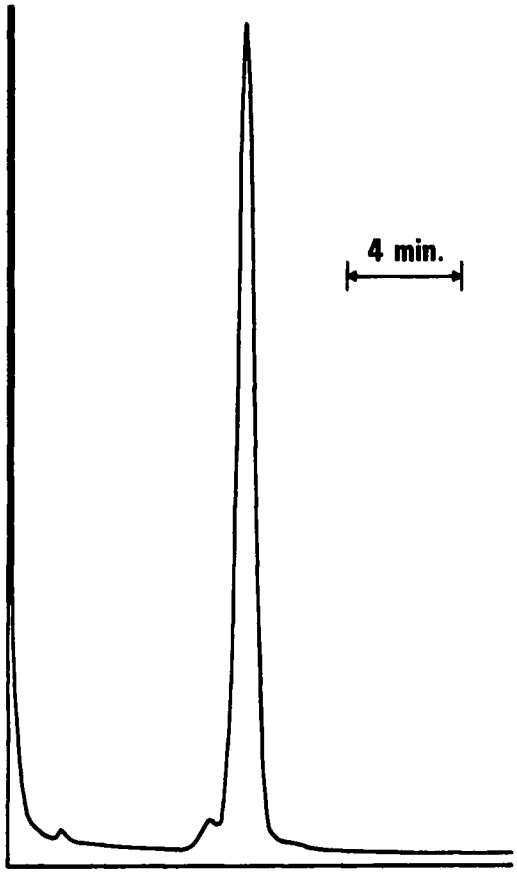

Figure 1—*Gas chromatogram of lincomycin 2-palmitate as the tris-O-trimethylsilyl derivative at 290°.*

stable under alkaline conditions; and in a few cases where crystallization failed to remove the impurities, the esters were extracted from mildly acidic, aqueous solutions with ether. The free base form of the ester in the organic phase was promptly converted to the stable hydrochloride salt.

TLC and GLC analyses of the esters showed only minor impurities in the final products. The GLC of lincomycin 2-palmitate as the *tris-O*-trimethylsilyl ether (Fig. 1) showed a trace impurity at 2 min. The small peak (7.1 min.) preceding the major peak is probably the 2-palmitate ester of lincomycin B (the 4′-ethyl analog of lincomycin), since a similar peak is seen in the GLC of the starting material (1) (5). The isomeric lincomycin 3- and 4-monopalmitates can be separated from lincomycin 2-palmitate, and these results will be reported later.

Bioactivity—The standard curve plate antibacterial assay using *Sarcina lutea* (UC 130) showed maximal activity with C_4–C_8 esters (Fig. 2). It is not clear whether these esters are intrinsically active or whether hydrolysis on the plate accounts for the activity. The effect of ester chain length on the oral CD_{50} (medium protective dose) in mice challenged with *Staphylococcus aureus* (UC 76) showed

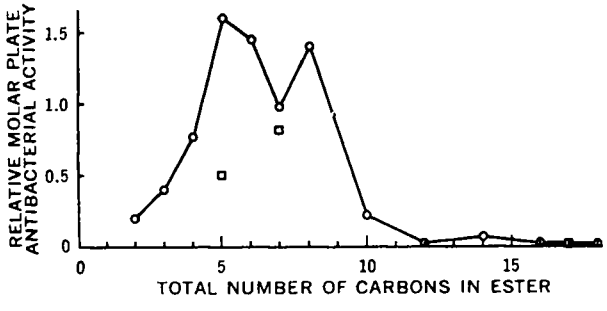

Figure 2—*Ratio of the molar plate antibacterial activity of lincomycin 2-esters to that of lincomycin using* S. lutea *(lincomycin = 1). Key: ○, acyl esters; and □, carbonate esters.*

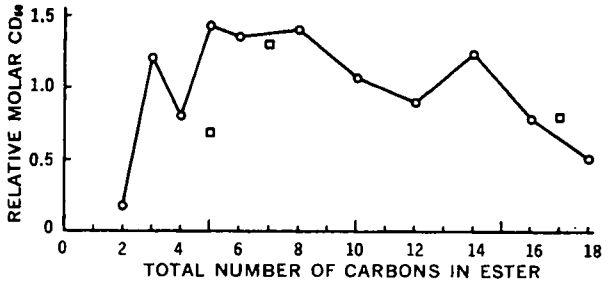

Figure 3—*Ratio of the molar oral CD_{50} of lincomycin 2-esters to that of lincomycin in mice infected with* Staph. aureus *(lincomycin = 1). Key: ○, acyl esters; and □, carbonate esters.*

that good activity was obtained with esters of chain lengths from C_4 to C_{16} (Fig. 3). The subcutaneous CD_{50} results (Fig. 4) were somewhat erratic but, in general, the results showed that the medium-chain length esters had better activity than the short-chain or the long-chain esters. The bioactivity of the carbonate esters was slightly less than the corresponding acyl esters.

Taste and Solubility—The water solubility of the hydrochloride salts of the lincomycin 2-acyl esters approaches zero above C_{12} (Fig. 5). Esters with chain lengths of C_{12} or greater are essentially tasteless. The laurate ester (C_{12}) is remarkable, since it is rather soluble and yet almost tasteless. The solubility of the esters, calculated (6) at pH 6.5, approaches zero above C_6 (Fig. 5). The lack of taste with the moderately water-soluble laurate ester can probably be explained by precipitation of the free base form of the ester in the mouth, since the pH of saliva is approximately 6.5 (7).

The 2-palmitate ester and the 2-*n*-hexadecylcarbonate ester of I have the desired properties of good CD_{50} activity and very low water solubility, indicating potentially good chemical stability in aqueous suspensions, and they are tasteless.

EXPERIMENTAL

Chromatography—TLC was conducted on silica gel G using the solvent system of hexane–ether–2-pentanone–methanol–concentrated ammonium hydroxide (60:20:20:9:1). The compounds were located by spraying the plates with ammonium sulfate followed by heating to about 180°.

GC was conducted after conversion of the lincomycin 2-acyl esters to the corresponding 3,4,7-*tris-O*-trimethylsilyl ethers, as described by Houtman *et al.* (5). A 1% solution of the ester in pyridine containing 20% hexamethyldisilazane and 20% trimethylchlorosilane was used to give the silyl ethers. After a 5-min. reaction period, 1 μl. of the solution was injected into a flame-ionization gas chromatograph[1] using a 0.61 × 33-cm. (0.125 × 17-in.) stainless steel column containing 3% OV-1 on Gas Chrom Q (100–120 mesh), with an oven temperature of 235–290°, depending on the chain length of the ester.

Synthesis—Lincomycin 2-esters were synthesized by reaction of an acid chloride, an acid anhydride, or a chlorocarbonate with II in

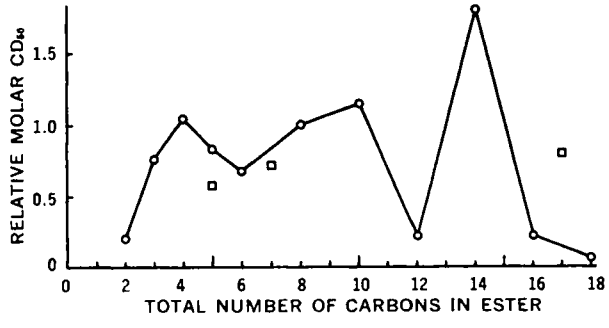

Figure 4—*Ratio of the molar subcutaneous CD_{50} of lincomycin 2-esters to that of lincomycin in mice infected with* Staph. aureus *(lincomycin = 1). Key: ○, acyl esters; and □, carbonate esters.*

[1] F & M model 700.

Table I—Analytical Data on Lincomycin 2-Monoesters Hydrochloride

2-Ester	Empirical Formula	Molecular Weight	Analytical Data[a] Calc.	Found	Water, %	Yield, %	Melting Point
n-Butyrate	$C_{22}H_{41}ClN_2O_7S$	513.10	C 51.50	51.43	4.88	45	185–187°
			H 8.05	7.93			
			Cl —	—			
			N 5.46	5.40			
			S 6.25	5.92			
n-Valerate	$C_{23}H_{43}ClN_2O_7S$	527.13	C 52.41	52.65	5.50	86	155–158°
			H 8.22	7.87			
			Cl 6.73	6.90			
			N 5.31	5.62			
			S 6.08	6.12			
n-Hexanoate	$C_{24}H_{45}ClN_2O_7S$	541.16	C 53.27	54.08	5.13	88	145–147°
				53.66	4.99		
			H 8.38	8.26			
			Cl 6.55	6.67			
			N 5.18	5.26			
			S 5.93	5.92			
n-Heptanoate	$C_{25}H_{47}ClN_2O_7S$	555.18	C 54.54	53.48	2.39	22	—[b]
			H 8.53	8.55			
			Cl 6.39	6.60			
			N 5.05	4.69			
			S 5.78	5.81			
n-Octanoate	$C_{26}H_{49}ClN_2O_7S$	569.22	C 54.86	54.83	2.93	50	—[b]
			H 8.68	8.68			
			Cl 6.23	6.56			
			N 4.92	4.97			
			S 5.63	5.86			
n-Decanoate	$C_{28}H_{53}ClN_2O_7S$	597.27	C 56.31	55.54	2.84	84	—[b]
				55.84	6.18		
			H 8.94	8.78			
			Cl 5.94	6.12			
			N 4.70	4.38			
			S 5.37	5.60			
n-Laurate	$C_{30}H_{57}ClN_2O_7S$	625.27	C 57.62	57.73	2.69	31	111–112.5°
			H 9.19	9.06			
			Cl —	—			
			N 4.48	4.67			
			S 5.13	5.13			
n-Myristate	$C_{32}H_{61}ClN_2O_7S$	653.33	C 58.83	58.32	2.89	69	139–141°
				58.67	3.22		
			H 9.41	9.08			
			Cl 5.43	5.70			
			N 4.29	4.65			
			S 4.91	4.93			
n-Palmitate	$C_{34}H_{65}ClN_2O_7S$	681.43	C 59.93	58.80	1.76	51	116–129°
				59.80	1.10		
			H 9.62	9.74			
			Cl 5.20	5.19			
			N 4.11	4.06			
			S 4.71	4.89			
n-Stearate	$C_{36}H_{69}ClN_2O_7S$	709.43	C 60.95	61.10	2.59	76	171–173.5°
			H 9.80	9.65			
			Cl —	—			
			N 3.95	4.03			
			S 4.52	4.36			
n-Butylcarbonate	$C_{23}H_{43}ClN_2O_8S$	543.14	C 50.86	51.15	3.07	54	—[b]
			H 7.98	8.10			
			Cl 6.53	6.52			
			N 5.16	5.32			
			S 5.90	6.05			
n-Hexylcarbonate	$C_{25}H_{47}ClN_2O_8S$	571.18	C 52.57	52.17	3.58	47	—[b]
			H 8.29	8.53			
			Cl 6.21	6.06			
			N 4.90	4.73			
			S 5.61	5.71			
n-Hexadecyl-carbonate	$C_{35}H_{67}ClN_2O_8S$	711.45	C 59.09	58.28	3.99	46	—[b]
			H 9.49	9.48			
			Cl 4.98	4.85			
			N 3.94	4.20			
			S 4.51	4.47			

[a] Combustion analyses and water determination (Karl Fischer) were conducted at The Upjohn Co. [b] The melting points reported were determined on a Thomas-Hoover capillary melting-point apparatus. The crystals appear to go through several stages of melting to give a wide melting range. The exact melting points are indefinite, since in most cases the compounds darken before melting. GLC showed that all compounds had a purity greater than 95% as estimated by area measurements.

pyridine. Representative examples of the synthesis of acyl esters and carbonate esters are described here, and the other esters were synthesized by similar methods. The analytical data are reported in Table I.

Method A: Esterification with Acyl Chlorides (Lincomycin 2-Stearate Hydrochloride)—A solution of 23.01 g. (0.03 mole) II in 100 ml. pyridine was treated with a solution of 12.12 g. (0.04 mole) stearoyl chloride in 40 ml. chloroform. After 15 min., 10 ml. of

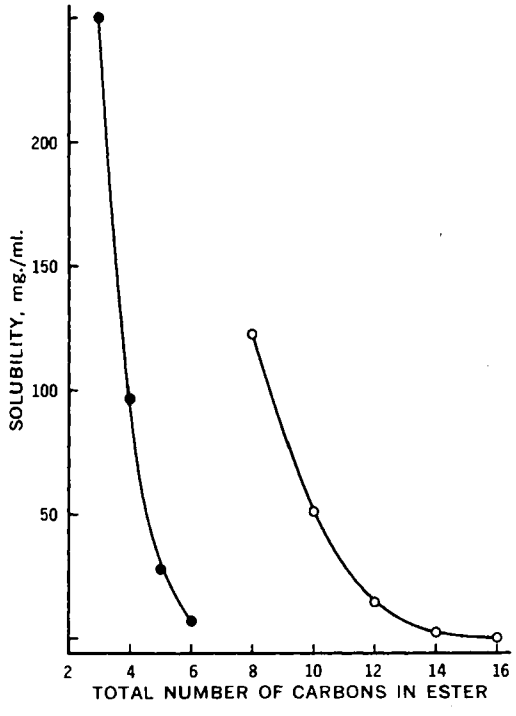

Figure 5—*Aqueous solubility of lincomycin 2-acyl esters* versus *total number of carbons in ester group. Key:* ○, *solubility of the hydrochloride salts of the esters; and* ●, *solubility of the esters calculated at pH 6.5.*

methanol was added and the solvent was removed under vacuum at 60°. The residual yellow semisolid was dissolved in 200 ml. 80% aqueous acetic acid, and the solution was heated on a steam bath for 25 min. Tritanol was removed by filtration after cooling the solution to room temperature, and the filtrate was taken to dryness under vacuum at 60°. The residue was dissolved in 120 ml. 80% aqueous acetone with heat, and the solution was diluted with 250 ml. acetone. The resulting white crystals (18.8 g., 76%) were isolated by filtration and air dried.

Method B: Esterification with Acid Anhydrides—A 2:10 mole ratio of acid anhydride to II was normally used. The reactions were conducted at room temperature or on a steam bath for 5–30 min. After removal of the protective groups, a slight excess of aqueous hydrochloric acid was added to an acetone solution of the ester to give the crystalline hydrochloride salts.

Method C: Esterification with Chlorocarbonates (Lincomycin 2-n-Butylcarbonate Hydrochloride)—A cold (−35°) solution of II (23.5 g.) in 200 ml. pyridine was treated with 5 g. *n*-butyl chlorocarbonate. The stirred suspension was allowed to warm to room temperature, then recooled to −35°, and treated with an additional 15 g. *n*-butyl chlorocarbonate. After warming to room temperature, the suspension was poured into 2 l. cold (0°) 0.01 *N* aqueous hydrochloric acid. The resulting precipitate was dissolved in 1 l. ether, and the phases were separated. The ether was removed under vacuum, and the residue was dissolved in a mixture of 1 l. methanol and 75 ml. water. The solution was adjusted to pH 1.0 with concentrated hydrochloric acid, and after 3 hr. at room temperature the solution was adjusted to pH 3 by addition of sodium bicarbonate. The solvent was removed under vacuum, and the residue was dissolved in 150 ml. chloroform. The chloroform solution was extracted twice with 150 ml. water. The combined aqueous layers were freeze dried to give 9 g. (54%) of white amorphous product.

Solubility Determinations—The solubility of the hydrochloride salts of the esters was determined at 24 ± 1° using excess compound equilibrated with water for 72 hr. Aliquots of the filtrates were evaporated to dryness at 120°, and the residue was weighed.

The solubility of the free base forms of the esters was determined by titration of aqueous solutions of the hydrochloride salts of the esters (1%) with 0.1 *N* aqueous sodium hydroxide. The solutions were filtered through a Millipore filter (1.5 μ) before titration. Titration was followed by periodically measuring the absorbance in a 1-cm. cell at 400 nm. At a certain point during titration, precipitation of the oily free base form of the ester occurred with a concomitant rapid increase in absorbance at 400 nm. Hypersaturation (8) did not occur, since there was no pH drop at the point of precipitation. The amount of base required to produce an absorbance of 0.10 divided by the volume of the solution gave the intrinsic solubility. The solubility at pH 6.5 was calculated (6):

$$S_0 = S_i[1 + \text{antilog}(\text{pKa} - \text{pH})] \qquad \text{(Eq. 1)}$$

where S_0 = solubility at pH 6.5, S_i = intrinsic solubility of the free base form, and pH = pH at which precipitation of the free base occurred.

The solubility determinations were conducted in duplicate, and the results were averaged. Repeat analyses differed by no more than 10%.

NMR Spectra at 60 MHz.—The NMR spectral features were similar to those reported previously (9). The long-chain esters such as the palmitate ester required probe temperatures of 75° to obtain well-resolved spectra.

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