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RESEARCH ARTICLES

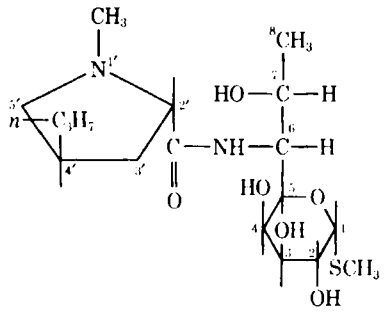
Chemical Modification of Lincomycin: Synthesis and Bioactivity of Selected 2,7-Dialkylcarbonate Esters

A. A. SINKULA▲ and C. LEWIS

Abstract □ A series of lincomycin 2,7-dialkylcarbonate esters was synthesized to enhance the pediatric acceptability of lincomycin. Several diester derivatives are sufficiently tasteless to warrant consideration as candidates for pediatric formulations. Preliminary bioactivities [mouse median protective dose (CD_{50}) bioassay] indicated several derivatives to be equivalent in subcutaneous activity to lincomycin hydrochloride. Four diester derivatives exhibited oral bioactivity comparable to that of lincomycin. Serum hydrolysis studies on certain 2,7-diesters of lincomycin established that a high degree of esterase activity is present in the serum of several different rodent species. This phenomenon appeared to be limited to these species.

Keyphrases □ Lincomycin 2,7-dialkylcarbonate esters—synthesis as tasteless derivatives, bioactivity compared to lincomycin hydrochloride □ Carbonate esters of lincomycin—synthesis as tasteless derivatives, bioactivity compared to lincomycin hydrochloride □ Pediatric formulations, potential—synthesis, activity of lincomycin 2,7-dialkylcarbonate esters □ Tasteless lincomycin derivatives—synthesis, activity of 2,7-dialkylcarbonate esters □ Antibacterial agents, potential—synthesis of lincomycin 2,7-dialkylcarbonate esters

The use of bioreversible derivatives for the modification of certain pharmaceutical properties of lincomycin (I) was reported previously (1, 2). This paper represents a continuation of that systematic effort and is concerned with lincomycin 2,7-dialkylcarbonate ester derivatives. The goal of this work was the synthesis of several

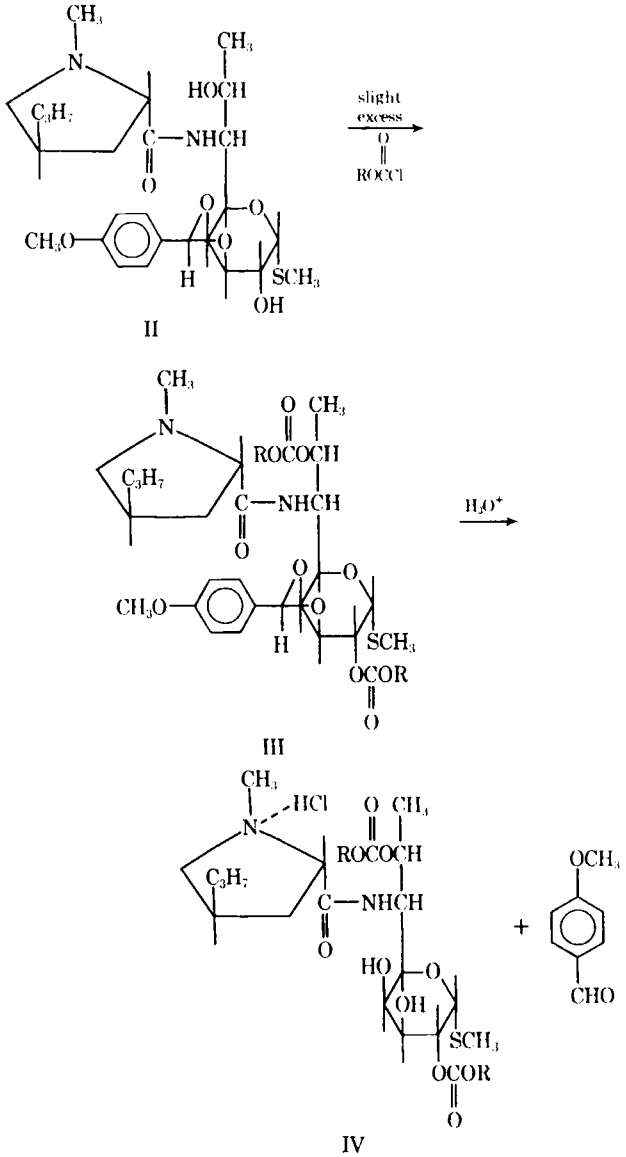


I

lincomycin 2,7-dialkylcarbonate esters sufficiently insoluble in aqueous media to warrant acceptability as candidates for a pleasant-tasting suspension of lincomycin for pediatric use.

RESULTS AND DISCUSSION

Chemistry—Lincomycin 2,7-dialkylcarbonate esters were synthesized according to Scheme I. The reaction of 3,4-*O*-(*p*-methoxybenzylidene)lincomycin (II) (3) with a slight excess (0.005–0.06 mole) of alkyl chloroformate afforded the desired 3,4-*O*-(*p*-methoxybenzylidene)lincomycin 2,7-dialkylcarbonate ester (III). Acidic hydrolysis of III produced lincomycin 2,7-dialkylcarbonate hydrochloride (IV) and anisaldehyde.



II

III

IV

Scheme I

Reaction temperature played an important role in the synthesis of III and IV. When the reaction mixture was cooled to 0° prior to addition of alkyl chloroformate, a pyridine–alkyl chloroformate complex precipitated which effectively prevented the formation of III. This situation prevailed even when the reaction mixture was recooled and more alkyl chloroformate was added. The problem was circumvented by cooling the reaction medium to −35° prior to addition of alkyl chloroformate. Subsequent slow warming of the reaction mixture to room temperature allowed formation of III exclusively.

When the formation of III was complete, the reaction mixture was poured into ice water and the pH was adjusted to 2–3 with concentrated hydrochloric acid. The resulting suspension was extracted with ether. Removal of the ether produced a yellow gum, which was dissolved in aqueous methanol acidified to pH 1–2. Hydrolysis of the *p*-methoxybenzylidene moiety proceeded smoothly. After hydrolysis was completed, the pH of the solution was adjusted to 4 with sodium bicarbonate. The solvent was removed *in vacuo* and the residue was extracted with ether. All derivatives were purified by recrystallization. Synthetic details are outlined in the *Experimental* section.

Bioactivity—*In Vitro*—The derivatives were all intrinsically inactive *in vitro* when tested by both the broth dilution and standard curve assay techniques (Table I). The methodology for determining *in vitro* bioactivity was described previously (3). In these assays, pure lincomycin base was assigned a potency of 1000 mcg./mg.

In Vivo—The antibacterial activities of the derivatives, as established in mice experimentally infected with *Staphylococcus aureus* (UC 76), are listed in Table II. All results were calculated as lincomycin base activity and are reported as the median protective dose

Table I—*In Vitro* Antibacterial Activity of Lincomycin 2,7-Dialkylcarbonate Derivatives by Broth Dilution and Standard Curve Assays[a]

Lincomycin Ester Hydrochloride	Activity Compared to Lincomycin Hydrochloride, MIC, mcg./mg.[b] Broth Dilution	Standard Curve
2,7-Dimethyl	<10 (<1)	1 (0.1)
2,7-Diethyl	<10 (<1)	1 (0.1)
2,7-Di-*n*-propyl	<10 (<1)	4 (0.4)
2,7-Di-*n*-butyl	<10 (<1)	5 (0.5)
2,7-Diisobutyl	<10 (<1)	1 (0.1)
2,7-Di-*n*-pentyl	<10 (<1)	5 (0.5)
2,7-Di-*n*-hexyl	<10 (<1)	6 (0.6)
2,7-Dicyclohexyl	~10 (1)	1 (0.1)
2,7-Dicyclohexylmethyl	~10 (1)	1 (0.1)

[a] Results expressed as micrograms of lincomycin base activity per milligram of ester and as percent of lincomycin base activity (in parentheses). [b] Pure lincomycin base = 1000 mcg./mg. MIC = minimal inhibitory concentration.

Table II—*In Vivo* Bioactivity of Lincomycin 2,7-Dialkylcarbonates[a] in Mice Experimentally Infected with *Staphylococcus aureus*

Lincomycin Ester Hydrochloride	Relative Median Protective Dose (CD_{50})[b] Subcutaneous	Oral
2,7-Dimethyl	0.53	0.59
2,7-Diethyl	0.96	0.86
2,7-Di-*n*-propyl	0.80	0.50
2,7-Di-*n*-butyl	1.04	0.65
2,7-Diisobutyl	0.75	0.40
2,7-Di-*n*-pentyl	1.66	0.51
2,7-Di-*n*-hexyl	2.35	0.83
2,7-Dicyclohexyl	0.68	0.04
2,7-Dicyclohexylmethyl	0.40	0.04

[a] Activities calculated as lincomycin base equivalents. [b] Median protective dose relative to that of lincomycin (lincomycin = 1.0). Mice challenged with approximately 100 lethal doses of *S. aureus*.

Table III—Hydrolysis of Lincomycin 2,7-Dialkylcarbonates in Serum from Various Animal Species

Serum Source	Di-carbonate Derivative	Lincomycin Activity[a], mcg./ml. 0 hr.	3 hr.	6 hr.	24 hr.
Mouse	Dipentyl	6.0	7.0	6.5	5.9
	Dihexyl	5.2	5.6	7.0	5.9
Rat	Dipentyl	3.3	5.1	5.5	6.0
	Dihexyl	3.8	5.1	5.5	6.0
Gerbil	Dipentyl	4.0	3.1	4.7	5.2
	Dihexyl	3.1	5.2	4.0	5.2
Rabbit	Dipentyl	0	6.5	5.8	6.4
	Dihexyl	0	3.0	4.5	4.8
Dog	Dipentyl	0	0	0	0
	Dihexyl	0	0	0	0
Monkey	Dipentyl	0	0	~0.32	1.8
	Dihexyl	0	0	0	1.0
Human	Dipentyl	0	0	0	~0.32
	Dihexyl	0	0	0	0
pH 7.0 buffer, no serum	Dipentyl	0	0	0	0
	Dihexyl	0	0	0	0

[a] Expressed as the MIC at the time of sampling. Limit of assay = 0.32 mcg./ml.; *i.e.*, 0 value = <0.32 mcg./ml. Original sample contained a theoretical 7-mcg./ml. (base equivalent) aliquot before hydrolysis.

(CD_{50}) in milligrams per kilogram per day. Also, a comparison to lincomycin was made with each determination, and the activity of the ester was expressed as the ratio between the bioactivity of lincomycin (considered as 1) and that of the derivative. Median protective doses (CD_{50}'s) observed with the subcutaneous route of administration demonstrated that the derivatives were absorbed and hydrolyzed readily by the mouse. Orally, good CD_{50} bioactivities were shown with the dimethyl, diethyl, di-*n*-butyl, and dihexyl esters. The di-*n*-propyl, diisobutyl, and dipentyl esters did not protect the infected animals as well as did lincomycin. The dicyclohexyl and dicyclohexylmethyl esters afforded virtually no protection to the infected animals.

Several diester derivatives were subjected to *in vitro* hydrolysis using serum from various animal species. They were tested at 10 mcg./ml. (about 7 mcg./ml. lincomycin base equivalent, depending on the derivative) at 37° in 50% fresh serum diluted in pH 7.0, 0.05 *M* tromethamine buffer. Samples were taken for testing at various times over 24 hr. At the end of each sampling time, the enzymes present in the serum were inactivated by immersing the sample in boiling water for 10 min. All derivatives were stable to boiling at pH 7.0 for greater than 20 min., and no derivative was hydrolyzed at 37° in pH 7 tromethamine buffer in 24 hr. In those instances where serum hydrolyzed the derivative, boiled serum was inactive. Samples were assayed for lincomycin bioactivity with the *Sarcina lutea*-sensitive assay (4).

Table III lists results with the dipentyl and dihexyl derivatives of lincomycin in mouse, rat, gerbil, monkey, dog, rabbit, and human serum. The striking activity of rodent serum is obvious. In these tests, the zero-time sample approximated a 5-min. sample, and all rodent serum exhibited hydrolytic activity in this short time.

Table IV summarizes the results from experiments in which the diethyl, dipropyl, di-*n*-butyl, dicyclohexyl, and dicyclohexylmethyl derivatives were exposed to the hydrolytic action of rat and human serum. Again, the high esterase activity for these derivatives was apparent in rat serum. Very little, if any, activity was exhibited by human serum.

EXPERIMENTAL

Typical reaction conditions are illustrated for the synthesis of lincomycin 2,7-dialkylcarbonate esters. Analytical data are listed in Table V.

Lincomycin 2,7-Dimethylcarbonate Hydrochloride—Anisylidene lincomycin base (II) (21 g., 0.04 mole) was dissolved in 200 ml. of pyridine and placed in a 500-ml. round-bottom flask fitted with an overhead paddle stirrer, a calcium chloride drying tube, and a dropping funnel. The solution was cooled to −35° using a dry ice–acetone bath. Methyl chloroformate (8 g., 0.08 mole) was added dropwise with stirring. Upon completion of addition, the reaction flask was slowly warmed to room temperature. TLC [silica gel[1] and a solvent system of hexane–ether–pentan-2-one–methanol–concentrated ammonium hydroxide (60:20:20:9:1)] indicated formation of predominately a lincomycin monomethylcarbonate along with some lincomycin dimethylcarbonate. The reaction mixture was again cooled to −35° and an additional 10 g. of methyl chloroformate was added dropwise. A second thin-layer chromatogram indicated that the reaction was not complete. The cooling procedure was again repeated and 15 g. more of methyl chloroformate was added. The reaction was again slowly warmed to room temperature. TLC showed that reaction was essentially complete.

Table IV—Hydrolysis of Lincomycin 2,7-Dialkylcarbonates by Human and Rat Serum

Serum Source	Dicarbonate Derivative	Lincomycin Activity, mcg./ml.[a] 0 hr.	3 hr.	24 hr.
Rat	Diethyl	6.4	7.0	8.4
	Dipropyl	6.0	7.5	7.0
	Di-*n*-butyl	7.5	8.2	7.5
	Dicyclohexal	1.1	4.5	4.6
	Dicyclohexylmethyl	5.4	8.2	7.5
Human	Diethyl	0	0	1.1
	Dipropyl	0	0	1.2
	Di-*n*-butyl	0	0	0.8
	Dicyclohexyl	0	0	0
	Dicyclohexylmethyl	0	0	0

[a] Activity expressed as the MIC in micrograms per milliliter. Limit of assay = 0.32 mcg./ml.; *i.e.*, 0 value = <0.32 mcg./ml. Original sample contained a theoretical ~7-mcg./ml. (base equivalent) aliquot before hydrolysis.

The mixture was poured into 1500 ml. of ice water which had been acidified to pH 2 with hydrochloric acid. About 1500 ml. of ether was used to extract the reaction product from water. Two hundred milliliters of ethanol was also added to ensure complete solubility of the precipitate in ether. The organic layer was evaporated on a steam bath. The resulting residue was dissolved in 100 ml. of acetone, and ether was added to the cloud point. The anisaldehyde which separated was decanted, and the ether–acetone solution was allowed to stand at room temperature for 2 days. The crystals that formed were collected and washed with acetone and then ether and air dried.

Lincomycin 2,7-Di-*n*-propylcarbonate Hydrochloride—Anisylidene lincomycin base (II) (31.5 g., 0.06 mole) was dissolved in 350 ml. of analytical reagent grade pyridine. The solution was cooled, with stirring, to −35° in a dry ice–acetone bath. *n*-Propyl chloroformate (22.06 g., 0.18 mole) was added dropwise from a dropping funnel to the cooled pyridine solution. The temperature of the reaction mixture was kept at −30 to −35° until all of the chloroformate had been added.

The resulting clear solution was stirred for 2 hr. at room temperature. TLC showed that reaction was complete. The reaction mixture was then poured into 2 l. of ice water, and the pH was adjusted to 3 with concentrated hydrochloric acid. The resulting aqueous suspension was extracted with two 500-ml. portions of ether, the ether layer was washed with 300 ml. of water and dried with anhydrous magnesium sulfate, and the ether was removed on a steam bath. A light-yellow syrup (47 g.) was obtained.

The syrup was dissolved in a mixture of 300 ml. of absolute methanol and 100 ml. of water and the pH of the solution was adjusted to 0.9 with concentrated hydrochloric acid. After 2 hr., sodium bicarbonate solution was added to raise the pH of the solution to 3.2. The methanol was removed under vacuum, and the resulting aqueous syrup was extracted with 100 ml. of ether–chloroform (1:1). The ether–chloroform layer was dried with magnesium sulfate, and the solvent was removed by warming on a steam bath. The resulting syrup was dissolved in 200 ml. of chloroform, and anhydrous acetone was added to the cloud point; this mixture was allowed to stand overnight in a refrigerator, resulting in crystallization of the product.

Lincomycin 2,7-Dicyclohexylcarbonate Hydrochloride: Cyclohexyl Chloroformate To 85 g. of liquid phosgene in a dry ice–

[1] Silica gel GF Uniplate, Analtech, Inc., Newark, Del.

Table V—Analytical Data: Lincomycin 2,7-Dialkylcarbonates

Lincomycin Ester Hydrochloride	Empirical Formula	Molecular Weight	Analysis (Corrected for Water Content), %— Calc.	Found	Melting Point[a]	Yield, %
2,7-Dimethyl	$C_{32}H_{39}ClN_2O_{10}S$	559.08	C 47.26	46.72	187–189°	15
			H 7.03	7.25		
			Cl 6.34	6.19		
			N 5.01	5.68		
2,7-Diethyl	$C_{34}H_{43}ClN_2O_{10}S$	587.13	C 49.10	49.43	196–197°	20
			H 7.38	7.51		
			Cl 6.04	6.18		
			N 4.77	4.71		
2,7-Di-*n*-propyl	$C_{36}H_{47}ClN_2O_{10}S$	615.17	C 50.76	50.58	202–203°	78
			H 7.70	7.99		
			Cl 5.76	5.74		
			N 4.55	4.47		
2,7-Di-*n*-butyl	$C_{38}H_{51}ClN_2O_{10}S$	643.24	C 52.28	52.36	204–205°	35
			H 7.99	8.07		
			Cl 5.51	5.65		
			N 4.36	4.58		
2,7-Diisobutyl	$C_{38}H_{51}ClN_2O_{10}S$	643.24	C 52.28	51.95	213–214°	30
			H 7.99	8.16		
			Cl 5.51	5.50		
			N 4.36	4.56		
2,7-Di-*n*-pentyl	$C_{40}H_{55}ClN_2O_{10}S$	671.28	C 53.68	53.43	221–223° dec.	75
			H 8.26	8.33		
			Cl 5.28	5.36		
			N 4.17	4.20		
2,7-Di-*n*-hexyl	$C_{42}H_{59}ClN_2O_{10}S$	699.35	C 54.96	55.26	218–219° dec.	74
			H 8.50	8.84		
			Cl 5.07	5.24		
			N 4.01	4.18		
2,7-Dicyclohexyl	$C_{42}H_{55}ClN_2O_{10}S$	695.32	C 55.28	54.62	185–186°	41
			H 7.97	7.87		
			Cl 5.10	5.05		
			N 4.03	4.21		
2,7-Dicyclohexylmethyl	$C_{44}H_{59}ClN_2O_{10}S$	723.37	C 56.45	56.10	223–226° dec.	69
			H 8.22	8.16		
			Cl 4.90	4.93		
			N 3.87	4.25		
3,4-*O*-(*p*-Methoxybenzylidene)-lincomycin	$C_{26}H_{40}N_2O_7S$	524.67	C 59.52	59.60	133–135°	75
			H 7.68	7.88		
			N 5.34	5.48		

[a] Melting points are of hydrated samples and were determined on a Thomas-Hoover melting-point apparatus and are uncorrected.

acetone bath was added, dropwise with stirring, 100 g. (1 mole) of cyclohexanol. The temperature of the reaction was maintained at about −20°. Upon completion of the cyclohexanol addition, the reaction was slowly warmed to room temperature. During warming, the mixture was stirred well to remove excess phosgene. After bubbling ceased, the reaction was connected to a water aspirator and the last traces of phosgene and hydrogen chloride were removed. The mixture was then aerated for 20 min. with nitrogen. The chloroformate was distilled at 27–31°/100 μ [lit. (6) 46–47°/2–3 mm.].

Anisylidene lincomycin base (II) (31.5 g., 0.06 mole) was dissolved in 350 ml. of analytical reagent grade pyridine and cooled to −35°. Cyclohexyl chloroformate (29.3 g., 0.18 mole) was added dropwise to this cooled solution. As the solution was slowly warmed, a precipitate formed. This precipitate was filtered, and 15 g. of cyclohexyl chloroformate was added to the recooled reaction mixture. The solution turned deep red as it warmed to room temperature. The mixture was stirred for 12 hr., after which time ice water was added and the mixture was acidified to pH 3 with concentrated hydrochloric acid. The resulting gum was dissolved in 1000 ml. of ether and dried with anhydrous magnesium sulfate. Removal of the solvent resulted in a reddish-brown gum, which was dissolved in 300 ml. of absolute methanol. Then 100 ml. of water was added to this solution, and the pH was adjusted to 0.8 with concentrated hydrochloric acid. The solution was stirred for 2 hr. Removal of methanol under reduced pressure afforded an aqueous suspension, which was extracted with 150 ml. of chloroform. The chloroform layer was dried with anhydrous magnesium sulfate and the solvent was removed. The resulting syrup was allowed to stand in a refrigerator overnight, and the crystals were collected on a filter and washed with ether. The crystals were finely powdered, suspended in 250 ml. of acetone ether (1:4), filtered, and dried.

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