Chemical Modification of Clindamycin: Synthesis and Evaluation of Selected Esters

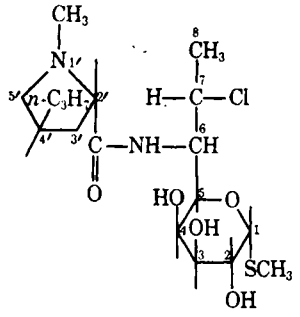
A. A. SINKULA▲, W. MOROZOWICH, and E. L. ROWE

Abstract □ Clindamycin was chemically modified to provide *in vivo* reversible derivatives that might suitably be utilized in special dosage forms. To improve taste properties, a series of 2- and 3-monoesters and some 2,3-dicarbonate esters of clindamycin were synthesized. A number of highly water-soluble salts were prepared in an attempt to provide an injectable intramuscular preparation with low incidence of pain. The long-chain clindamycin 2-monoesters (palmitate and hexadecylcarbonate) are virtually devoid of the characteristic bitter taste of clindamycin. The water-soluble salts (gluconate and lactobionate) of clindamycin showed no significant improvement over clindamycin in muscle irritation studies. Clindamycin blood levels were determined in dogs following oral administration of some esters. Clindamycin 2-palmitate and clindamycin 2-hexadecylcarbonate exhibited blood levels equivalent to clindamycin hydrochloride. The CD_{50} results using mice infected with *Staphylococcus aureus* showed that several esters possess antibacterial activity equivalent to that of clindamycin hydrochloride.

Keyphrases □ Clindamycin esters and salts—synthesis, bioactivity compared to hydrochloride salt, tasteless long-chain esters □ Antibacterial agents, potential—synthesis of clindamycin esters and salts, compared to hydrochloride salt □ Tasteless clindamycin derivatives—synthesis, blood levels in dogs and antibacterial activity in mice compared to hydrochloride salt □ Pediatric formulations, potential—synthesis, antibacterial activity of long-chain clindamycin esters

Clindamycin [7(S)-chloro-7-deoxylincomycin] (I) hydrochloride is a new, semisynthetic antibiotic having a high degree of antibacterial activity against Gram-positive organisms and a lower order of activity against Gram-negative organisms (1, 2). Its spectrum of activity approximates that of erythromycin and lincomycin (3). Clindamycin is well absorbed from the GI tract and affords serum levels approximately twice those of lincomycin (1, 4).

The fact that clindamycin is extremely bitter *per se* precludes its formulation as an acceptable pediatric syrup. Furthermore, the incidence of pain at the injection site is quite high on intramuscular administration of clindamycin hydrochloride. These factors prompted this investigation of the chemical modification of clindamycin for the purpose of enhancing its pharmaceutical acceptability. This article reports the synthesis and evaluation of selected 2- and 3-monoesters and 2,3-dicarbonate esters of clindamycin.



I: clindamycin

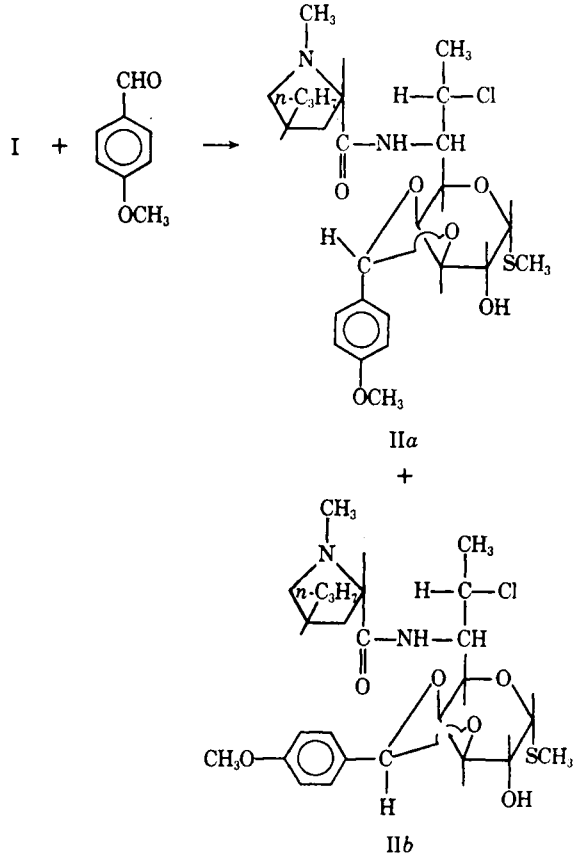
DISCUSSION

Chemistry—The synthesis of 2-esters of clindamycin was achieved by protection of the 3- and 4-hydroxyl groups of clindamycin (I) with the acid labile anisylidene moiety (5, 6). The reaction of anisaldehyde with I gave two spots by TLC, which were identified by NMR as the benzylic isomers II*a* and II*b* (Scheme I). Esterification of the 2-hydroxyl group of 3,4-*O*-anisylidene clindamycin (II), followed by treatment of the resulting ester acetal with acid, afforded pure clindamycin 2-acyl and 2-carbonate esters (Scheme II).

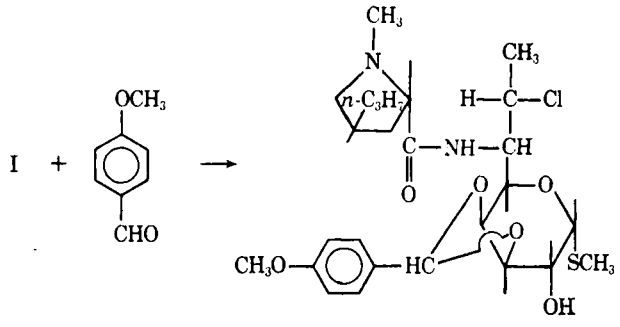
Clindamycin 3-pentylcarbonate was prepared in 54% yield by selective esterification of I in pyridine at low temperatures (−25°) (Scheme III) (7). Purification was achieved by selective solvent extraction under controlled pH conditions while purity was monitored by GLC (Figs. 1 and 2).

Clindamycin 2,3-dicarbonate esters (Scheme IV) were prepared by selective acylation of clindamycin in pyridine using excess acylating agent. The gluconate and lactobionate salts of clindamycin were prepared by mixing aqueous solutions of stoichiometric amounts of clindamycin base with either δ-gluconolactone or lactobiono-δ-lactone and then allowing them to stand for 1 hr., with subsequent removal of water *in vacuo*.

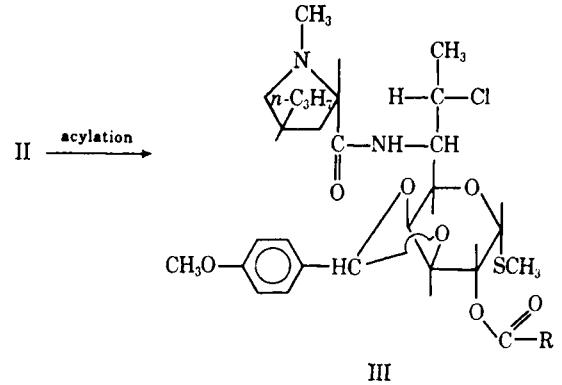
NMR—The 60-MHz. spectra of the benzylic isomers II*a* and II*b* in $CDCl_3$ showed the benzylic proton at 6.28 δ in one isomer and at



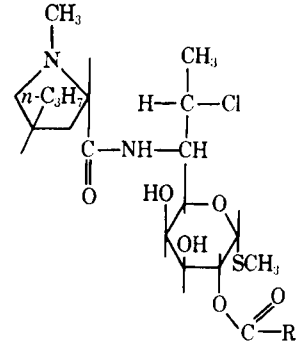
Scheme I—3,4-O-Anisylidene clindamycin



II



III



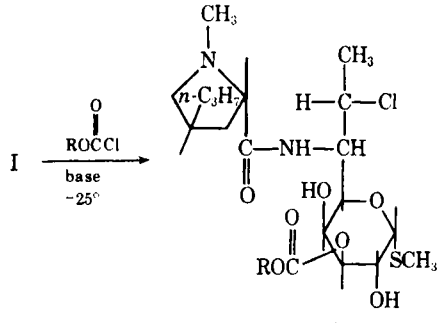
IV

Scheme II—Clindamycin 2-monoesters (R = alkyl and R = O-alkyl)

5.80 δ in the other (dimethylformamide-d_7). The isomer with a signal at 6.16 δ was assigned to II*b* since 3,4-*O*-anisylidene lincomycin shows the benzylic proton at 6.16 δ.

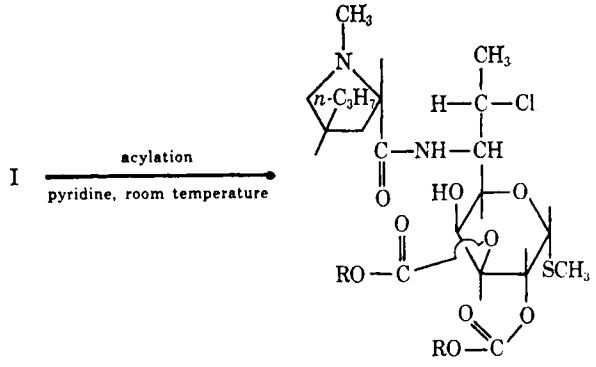
The 60-MHz. NMR spectra of the 2-clindamycin monoesters were similar to the spectra of the 2-monoesters of lincomycin and were thoroughly reviewed in previous reports (8, 9).

Bioactivity—The methods used for determining *in vitro* and *in vivo* bioactivities of clindamycin esters are the same as those used for lincomycin esters and were discussed previously (10).



54%

Scheme III—Clindamycin 3-monocarbonate esters (R = $-C_5H_{11}$)



Scheme IV—Clindamycin 2,3-dicarbonate esters

The bioactivities of the various clindamycin esters (both *in vitro* and *in vivo*) are summarized in Table I. The *in vitro* data show all esters to be less bioactive than clindamycin. This is to be expected since ester hydrolysis must occur to liberate free clindamycin. Many esterified antibiotics have been shown to be inactive until ester hydrolysis occurs (11, 12). In all probability, this is the situation with clindamycin 2-hexanoate, 2-hexylcarbonate, 2-cinnamate, and 3-pentylcarbonate. The above-mentioned short- to medium-chain length derivatives would be more soluble in the culture media than, for example, clindamycin 2-palmitate[1], 2-hexadecylcarbonate, or 2,3-dicarbonates. Increased solubility would increase the availability of enzymatic hydrolysis. This is apparent from the plate bioactivity data for the short- to medium-length chain compounds *versus* the longer chain derivatives.

The mouse subcutaneous *in vivo* data for the clindamycin 2-derivatives indicate that bioactivity is comparable to clindamycin up to and including an ester chain length of six carbons. The bioactivity decreased with esters containing 12 or more carbon atoms. Clindamycin 2-(*o*-benzoyl)benzoate bioactivity is low due probably to difficulty of hydrolysis of the sterically hindered ester. The clindamycin 2,3-dicarbonate esters possess low subcutaneous activity due to the probable low solubility of these compounds. The oral antibacterial activity of the esters roughly correlates with the subcutaneous activity pattern.

Dog serum levels for selected clindamycin esters and salts are shown in Table II. Many of the esters show an area under the blood level curve comparable to clindamycin hydrochloride, indicating high bioavailability.

Taste Studies—Four clindamycin 2-acyl esters of varying chain length (palmitate, laurate, hexanoate, and acetate) were dissolved in 30% sucrose solutions. The clindamycin ester solutions were coded A, B, C, and D, and 5-ml. samples of each were given to a taste panel of 26 people. The usual protocol was followed, with 1 hr. allowed between samples. The taste of each was rated on a scale of 1 to 9 (1 = poor, 9 = excellent). The raw score data were analyzed by computer to show any significant differences between samples.

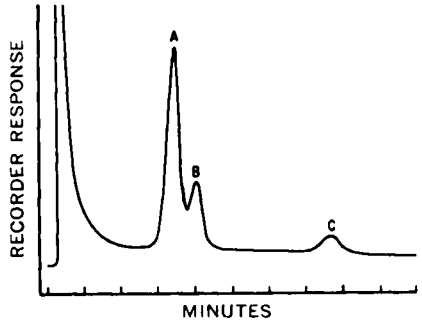


Figure 1—*Products of 7(S)-chloro-7-deoxylincomycin 3-pentylcarbonate reaction (see* Experimental *section). Key: A, 7(S)-chloro-7-deoxylincomycin 3-pentylcarbonate; B, 7(S)-chloro-7-deoxylincomycin 2-pentylcarbonate; and C, 7(S)-chloro-7-deoxylincomycin 2,3-dipentylcarbonate.*

[1] Available commercially as Cleocin Palmitate, The Upjohn Co., Kalamazoo, Mich.

Table I—Antibacterial Activity of Clindamycin 2- and 3-Monoesters and 2,3-Diesters[a]

Ester	Antibacterial Activity— *In Vitro* Activity, mcg./mg.[b]	Relative Median Protective Dose (CD_{50})[c] Subcutaneous	Oral
Clindamycin 2-hexanoate hydrochloride	690 (69)	1.31	0.67
Clindamycin 2-hexylcarbonate hydrochloride	330 (33)	1.36	0.43
Clindamycin 2-laurate hydrochloride	20 (2.0)	<0.1	<0.28
Clindamycin 2-palmitate hydrochloride	21 (2.1)	<0.3	0.21
Clindamycin 2-hexadecylcarbonate hydrochloride	<4 (<0.4)	<0.1	0.23
Clindamycin 2-(1-adamantoate) hydrochloride	9 (0.9)	1.26	0.81
Clindamycin 2-(*p*-benzoyl)benzoate hydrochloride	55 (5.5)	1.67	0.54
Clindamycin 2-(*o*-benzoyl)benzoate hydrochloride	7 (0.7)	<0.15	0.29
Clindamycin 2-cinnamate hydrochloride	260 (26)	1.73	2.33
Clindamycin 2-diphenylacetate hydrochloride	—[d]	—[d]	—[d]
Clindamycin 2-succinate	375 (37.5)	0.83	0.93
Clindamycin 3-pentylcarbonate hydrochloride	180 (18)	0.37	0.67
Clindamycin 2,3-dibutylcarbonate hydrochloride	<4 (<0.4)	0.39	0.80
Clindamycin 2,3-dipentylcarbonate hydrochloride	<4 (<0.4)	<0.1	0.20
Clindamycin 2,3-dihexylcarbonate hydrochloride	<4 (<0.4)	0.18	0.60
Clindamycin lactobionate	520 (52)	0.69	0.94
Clindamycin gluconate	620 (62)	0.63	1.05

[a] Activities calculated as clindamycin base equivalents. [b] As measured on a standard curve agar assay *versus Sarcina lutea*. Results expressed as micrograms of clindamycin base activity per milligram of ester and as percent of lincomycin base activity (in parentheses). [c] Median protective dose relative to that of clindamycin (clindamycin = 1.0) in the mouse. [d] Data not available.

The average scores shown in Fig. 3 indicate a linear trend of taste improvement with increasing chain length. The palmitate ester is significantly better than the laurate at the 5% level of confidence. The laurate, in turn, is significantly better than both the hexanoate and acetate. These latter two were ranked equally low, being very bitter-tasting compounds.

Clindamycin 2-palmitate hydrochloride is essentially tasteless, and dog blood level studies (Table II) showed that *in vivo* regeneration of clindamycin is quantitative. For these reasons, clindamycin 2-palmitate hydrochloride is being extensively tested in humans as a tasteless pediatric formulation.

EXPERIMENTAL

GLC was run on the silanized clindamycin esters. The silyl ethers were made by mixing a pyridine solution containing approximately 2% of the ester to be silanized, 20% trimethylchlorosilane, and 20% hexamethyldisilazane. One microliter of this solution was injected into a gas chromatograph[2] containing a flame-ionization detector. The column was 0.3 × 43.2-cm. (0.12 × 17-in.) stainless steel, containing 1% OV-1 on Gas Chrom Q (100–120 mesh). Oven temperatures ranged from 200 to 245° and were linearly programmed at 3°/min.

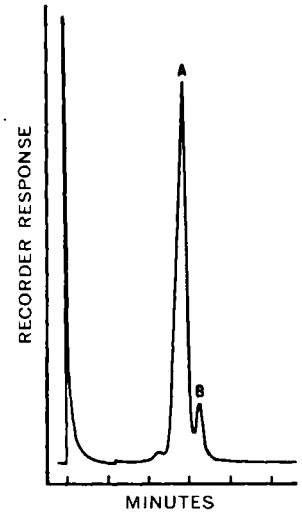


Figure 2—*Two-extraction purification of 7(S)-chloro-7-deoxylincomycin 3-pentylcarbonate. Key: A, 7(S)-chloro-7-deoxylincomycin 3-pentylcarbonate; and B, 7(S)-chloro-7-deoxylincomycin 2-pentylcarbonate.*

TLC was conducted on TLC plates[3] precoated with silica gel GF (250-μ thickness). A solvent system of hexane–ether–methyl propyl ketone–methanol–ammonia (60:20:20:9:1) (Solvent A) was used. Spots were developed with 20% aqueous ammonium sulfate, and the plate was then heated after spraying to char the organic material present.

Typical examples of reaction conditions for the synthesis of various clindamycin esters are illustrated. Analytical data are listed in Table III.

3,4-*O*-*p*-Anisylidene-7(S)-chloro-7-deoxylincomycin Hydrochloride—*Synthesis*—A solution of 65.0 g. 7(S)-chloro-7-deoxylincomycin hydrochloride hemihydrate dissolved in a mixture of 90 ml. dimethylformamide, 120 ml. anisaldehyde, and 250 ml. benzene was heated to 90°. The benzene–water azeotrope was allowed to distil at 90–95° and, upon collecting each 100 ml. distillate, an additional 100 ml. dry benzene was added. After the collection of 1000 ml. benzene, an extra 100 ml. dry benzene was added and some crystallization occurred. After 4500 ml. distillate was collected, an additional 500 ml. benzene was added. Fifty milliliters distillate was collected and the reaction mixture was allowed to cool to room temperature. The yellow reaction mixture became thick with crystals. The solids were isolated by filtration, washed with 100 ml. benzene–dimethylformamide (9:1), and finally washed with benzene. The resulting crystals were dried *in vacuo* at 45°.

Separation of Isomers by Preparative TLC—The isomers of 3,4-*O*-*p*-anisylidene-7(S)-chloro-7-deoxylincomycin were separated by preparative TLC, using silica gel G (0.25-mm. layers) with Solvent A. After development, the chromatograms were visualized with UV light and the isomers were scraped off the plate and eluted with methanol. The methanol extracts of the acetals were taken to dryness, yielding viscous tan liquids. The mass spectra of the two isomers were identical.

Clindamycin 2-Monoesters—*7(S)-Chloro-7-deoxylincomycin 2-Hexylcarbonate Hydrochloride*—3,4-*O*-Anisylidene-7(S)-chloro-7-deoxylincomycin hydrochloride (11.59 g., 0.02 mole) was dissolved in 100 ml. of analytical reagent grade pyridine, and the solution was cooled to −30°. Hexyl chloroformate[4] (6.29 g., 0.0382 mole) was added dropwise with good stirring. An additional 2 g. of hexyl chloroformate was added at 1-hr. intervals. The reaction was terminated after 3.5 hr. of stirring by pouring into 1 l. of ice water acidified to pH 2 with concentrated hydrochloric acid. The resulting aqueous suspension was extracted with three 250-ml. portions of chloroform, the chloroform extracts were combined and dried with anhydrous magnesium sulfate, and the chloroform was

[2] F & M model 700.

[3] Uniplates, Analtech, Inc.

[4] Eastman White Label.

Table II—Dog Serum Levels of Clindamycin Esters and Salts

Clindamycin Derivative	Dose, mg./kg. Clindamycin Base Equivalent	Serum Level, mcg./ml., as Clindamycin Base						Area[b] under Curve
		0.5 hr.	6 hr.	12 hr.	24 hr.	T_{50}	Peak Concentration[a]	
Clindamycin 2-hexanoate hydrochloride	25 (oral)	10.72	4.71	2.57	1.59	6.3	10.72 (0.5)	88.9
Clindamycin 2-hexylcarbonate hydrochloride	10 (i.m.)	0	0.59	0.5	<0.35	11.2	0.59 (4 and 6)	11.1
Clindamycin 2-laurate hydrochloride	25 (oral)	7.38	4.00	2.15	1.32	9.46	8.40 (2)	76.11
Clindamycin 2-palmitate hydrochloride	25 (oral)	7.19	4.05	2.21	1.34	9.39	8.29 (1)	76.09
Clindamycin 2-hexadecylcarbonate hydrochloride	25 (oral)	3.0	3.01	1.5	1.01	9.7	5.76 (2)	69.5
Clindamycin 2-(1-adamantoate) hydrochloride	25 (oral)	9.15	4.0	1.92	1.04	4.8	13.52 (1)	81.9
Clindamycin 2-(*p*-benzoyl)benzoate hydrochloride	25 (oral)	2.94	1.98	0.99	0.64	5.9	4.08 (2)	35.9
Clindamycin 2-(*o*-benzoyl)benzoate hydrochloride	25 (oral)	0	0.54	0.46	<0.38	10.5	0.65 (2)	10.5
Clindamycin 2-cinnamate hydrochloride	25 (oral)	4.1	2.33	1.58	0.97	7.1	5.44 (1)	48.4
Clindamycin 2-diphenylacetate hydrochloride	25 (oral)	1.45	3.07	1.65	1.25	8.2	4.03 (2)	50.3
Clindamycin 2-succinate	10 (i.m.)	4.11	2.0	0.96	0.7	5.4	4.69 (1)	38.8
Clindamycin 2,3-dibutylcarbonate hydrochloride	25 (oral)	3.09	3.89	2.15	1.31	6.8	7.85 (2)	71.6
Clindamycin 2,3-dihexylcarbonate hydrochloride	25 (oral)	0.5	0.38	0	0	3.55	0.67 (1)	3.95
Clindamycin lactobionate	10 (i.m.)	4.7	2.07	1.04	0.26	4.9	5.3 (1)	37.8
Clindamycin gluconate	10 (i.m.)	5.75	3.26	1.87	1.36	7.0	6.15 (1)	61.8
Clindamycin hydrochloride	25 (oral)	14.45	3.55	1.83	1.15	4.7	14.45 (0.5)	76.8
Clindamycin hydrochloride	10 (i.m.)	4.31	2.49	1.09	0.74	5.7	4.89 (2)	43.2

[a] Number in parentheses equals time in hours. [b] Area expressed as concentration × time.

removed under reduced pressure at 38°. The residue was dissolved in 100 ml. of methanol and 40 ml. of distilled water. The pH of the solution was adjusted to 1.5 with concentrated hydrochloric acid and stirred for 75 min. The pH was then lowered to 1, and the solution was stirred for 3.75 hr. Sodium bicarbonate solution was then used to adjust the solution to pH 3.5, and all solvent was removed under reduced pressure at 36°. Two hundred milliliters of chloroform was then added to precipitate the sodium chloride, and the solvent was removed at high vacuum at 60°. One hundred milliliters of acetone was added to precipitate the desired product, which was subsequently washed with two 50-ml. portions of ether. The yield was 8.85 g. (75%).

7(S)-Chloro-7-deoxylincomycin 2-Palmitate Hydrochloride—A solution of 23.18 g. (0.04 mole) of 3,4-*O*-*p*-anisylidene-7(S)-chloro-7-deoxylincomycin hydrochloride dissolved in 400 ml. dry pyridine and 40 ml. analytical reagent grade chloroform was treated with a solution of 24.38 g. (0.08 mole) of palmitoyl chloride dissolved in 50 ml. chloroform. The acid chloride solution was added dropwise to the stirred pyridine solution over 1 hr. The pale-orange solution was diluted with 100 ml. isopropyl alcohol, and the solvent was removed under high vacuum at 60°. Then the viscous orange residue was dissolved in 200 ml. hot glacial acetic acid, and the resulting solution was diluted with 40 ml. of water. The solution was heated on a steam bath for 15 min., and the solvent was removed under high vacuum at 60°. The orange residue was dissolved in 100 ml. isopropyl alcohol and evaporated as before. The orange residue was dissolved in 150 ml. hot acetone, and the solution was added dropwise to 1500 ml. acetonitrile while stirring. The resulting precipitate of 7(S)-chloro-7-deoxylincomycin 2-palmitate hydrochloride was isolated by filtration under nitrogen pressure. The cake was washed with acetonitrile and dried by passing nitrogen through the filter for 15 min. Final drying was carried out at room temperature under high vacuum. The yield was 23.5 g. (84%).

7(S)-Chloro-7-deoxylincomycin 2-(p-Benzoyl)benzoate Hydrochloride—The preparation of *p*-(benzoyl)benzoyl chloride is as follows. *p*-Benzoyl benzoate (6.78 g.), recrystallized from water, was placed in a 125-ml. conical flask. Enough thionyl chloride was then added to cover the white powder and form a thin slurry. Excess thionyl chloride was removed, along with evolved sulfur dioxide and hydrogen chloride gas, by heating on a steam bath. The pale-yellow crystals were dried overnight at 45°, and the yield was 5.5 g.

The esterification procedure used is as follows. 3,4-*O*-*p*-Anisylidene-7(S)-chloro-7-deoxylincomycin hydrochloride (5.8 g., 0.01 mole) was dissolved in 35 ml. pyridine. *p*-(Benzoyl)benzoyl chloride (2.94 g., 0.012 mole) was dissolved in 10 ml. chloroform and added dropwise to the stirred pyridine solution. About halfway through the addition, a white precipitate formed which remained until heating (~50°) caused it to redissolve. Subsequent cooling to room temperature caused no further precipitation. TLC indicated that the reaction was half complete after 4 hr. at room temperature. Another 1.5 g. *p*-(benzoyl)benzoyl chloride dissolved in 5 ml. chloroform was added dropwise, and the reaction flask was warmed to 45°. TLC showed the reaction to be 90% complete. After addition of 3 ml. water the solvents in the reaction flask were removed *in vacuo*. The residue was washed with three 100-ml. portions of isopropyl alcohol, which in turn was removed *in vacuo*. The light-yellow residue was dissolved in 40 ml. glacial acetic acid and heated on a steam bath. To this was added 10 ml. water, and the solution was heated for 40 min. The yellow solution was evaporated *in vacuo;* the residue was washed with 100 ml. isopropyl alcohol, 90 ml. isopropyl alcohol plus 15 ml. 1 *N* HCl, and then 100 ml. isopropyl alcohol again, with the solvents being removed *in vacuo*. The residue was dissolved in 50 ml. chloroform, and the clear solution was added slowly to 500 ml. of cold anhydrous ether. The white precipitate was filtered and dried under nitrogen. TLC showed essentially pure compound.

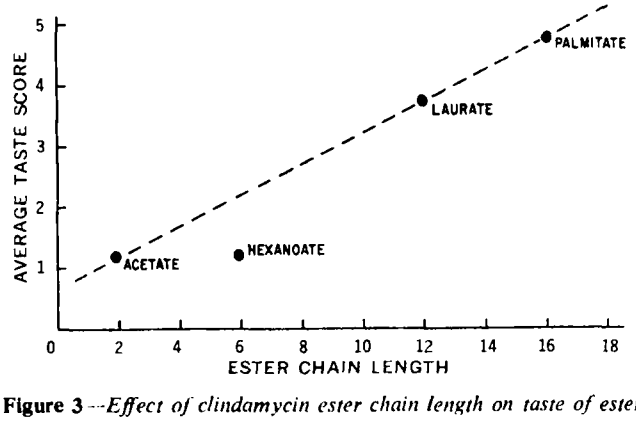


Figure 3—*Effect of clindamycin ester chain length on taste of ester in syrup.*

Table III—Analytical Data Obtained with Clindamycin Ester Hydrochlorides and Salts

Clindamycin Derivative	Empirical Formula	Molecular Weight	Analysis[a] Calc.	Found	Yield, %	Melting Point[b]
2-Hexanoate	$C_{24}H_{44}Cl_2N_2O_6S$	559.16	C 51.51	50.94	50	171–175°
			H 7.93	8.14		
			Cl 12.67	12.45		
			N 5.01	4.97		
			S 5.73	5.77		
2-Hexylcarbonate	$C_{25}H_{46}Cl_2N_2O_7S$	589.6	C 50.93	50.57	75	206–209° dec.
			H 7.86	7.84		
			Cl 12.03	12.16		
			N 4.75	4.50		
			S 5.44	5.26		
2-Laurate	$C_{30}H_{56}Cl_2N_2O_6S$	643.77	C 55.97	56.11	10	145–160°
			H 8.77	8.66		
			Cl 11.02	9.96		
			N 4.35	4.36		
			S 4.98	4.91		
2-Palmitate	$C_{34}H_{64}Cl_2N_2O_6S$	699.89	C 58.35	58.20	84	126° dec.
			H 9.22	9.15		
			Cl 10.13	9.91		
			N 4.00	3.81		
			S 4.85	4.46		
2-Hexadecylcarbonate	$C_{35}H_{66}Cl_2N_2O_7S$	729.87	C 57.60	57.34	38	190–193° dec.
			H 9.12	9.57		
			Cl 9.72	9.63		
			N 3.84	3.65		
			S 4.39	4.43		
2-(1-Adamantoate)	$C_{29}H_{48}Cl_2N_2O_6S$	623.66	C 55.85	55.62	45	159–162° dec.
			H 7.76	7.89		
			Cl 11.37	11.23		
			N 4.49	4.45		
2-(*p*-Benzoyl)benzoate	$C_{32}H_{42}Cl_2N_2O_7S$	669.68	C 57.39	56.55	57	214–216°
			H 6.32	6.11		
			Cl 10.59	10.35		
			N 4.18	4.24		
			S 4.79	4.51		
2-(*o*-Benzoyl)benzoate	$C_{32}H_{42}Cl_2N_2O_7S$	669.68	C 57.39	58.57	50	203–207°
			H 6.32	6.46		
			Cl 10.59	9.40		
			N 4.18	4.96		
			S 4.79	4.19		
2-Cinnamate	$C_{27}H_{40}Cl_2N_2O_6S$	591.0	C 54.83	54.98	85	170–172° dec.
			H 6.82	6.59		
			Cl 11.99	11.61		
			N 4.74	4.48		
			S 5.42	5.13		
2-Diphenylacetate	$C_{32}H_{44}Cl_2N_2O_6S$	655.70	C 58.62	58.40	75	206–208° dec.
			H 6.76	6.88		
			Cl 10.82	11.45		
			N 4.27	4.34		
			S 4.89	4.77		
2,3-Dibutylcarbonate	$C_{28}H_{50}Cl_2N_2O_9S$	661.69	C 50.83	50.92	38	205–207°
			H 7.62	8.32		
			Cl 10.72	11.21		
			N 4.23	4.23		
2,3-Dihexylcarbonate	$C_{32}H_{58}Cl_2N_2O_9S$	717.80	C 53.55	53.63	48	202–203°
			H 8.14	8.04		
			Cl 9.88	10.04		
			N 3.90	4.07		
3-Pentylcarbonate	$C_{24}H_{44}Cl_2N_2O_7S$	575.58	C 50.08	48.24	49	145–150°
			H 7.71	7.60		
			Cl 12.32	13.17		
			N 4.87	4.61		
3,4-*O*-*p*-Anisylidene-7(S)-chloro-7-deoxylincomycin hydrochloride	$C_{26}H_{40}Cl_2N_2O_6S$	579.61	C 53.88	53.76	75	155–161° dec.
			H 6.96	7.12		
			Cl 12.23	11.85		
			N 4.83	5.26		
			S 5.53	5.54		
Lactobionate salt	$C_{30}H_{55}ClN_2O_{17}S$	783.28	C 46.00	45.30	80	113–116°
			H 7.08	7.31		
			Cl 4.53	4.03		
			N 3.57	2.95		
Gluconate salt	$C_{24}H_{43}ClN_2O_{12}S$	621.13	C 46.41	45.54	80	73–75°
			H 7.30	7.51		
			Cl 5.71	5.26		
			N 4.51	4.16		

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

7(S)-Chloro-7-deoxylincomycin 2-(1-Adamantoate) Hydrochloride—3,4-*O*-*p*-Anisylidene-7(S)-chloro-7-deoxylincomycin hydrochloride (5.8 g., 0.01 mole) was dissolved in 35 ml. pyridine, and the solution was cooled to 4°. Then 2.4 g. (0.012 mole) adamantoyl chloride was dissolved in 10 ml. chloroform, and this was added dropwise to the stirred pyridine solution. The temperature was held between 5 and 8° during the addition and for 30 min. thereafter. TLC after 2 hr. indicated that 90% of the starting material had reacted. One gram adamantoyl chloride in 5 ml. chloroform was added dropwise to the pyridine solution (0–5°). After standing at room temperature overnight, the solvents were removed *in vacuo*.

The residue was treated with 80 ml. methanol and once with 80 ml. methanol plus 10 ml. 1 *N* HCl; each time the resulting solution was evaporated to dryness. The yellow gummy residue was dissolved in 40 ml. glacial acetic acid and heated on the steam bath for 5 min. Twenty milliliters water was added, and the solution was heated for another 30 min. The solvent was removed *in vacuo* as before, and the residue was washed three times with 80 ml. isopropyl alcohol, which was removed *in vacuo*. The product, a yellow oil, was dissolved in 40 ml. chloroform and extracted twice with 40-ml. portions of pH 2.2 buffer. The organic phase was dried (anhydrous sodium sulfate), made acidic with anhydrous hydrochloric acid (pH 1–2, pHydrion paper), and reduced to dryness. The residue was dissolved in 10 ml. chloroform and poured slowly into 500 ml. of stirred ether at room temperature. The white precipitate was isolated by filtration, washed with 75 ml. ether, and dried at 45° *in vacuo*.

Clindamycin 3-Monoesters [7(S)-Chloro-7-deoxylincomycin 3-Pentylcarbonate Hydrochloride]—A solution of 9.63 g. (0.02 mole) of 7(S)-chloro-7-deoxylincomycin hydrochloride in 100 ml. of pyridine was cooled to about −30°, and 6.02 g. (0.04 mole) of *n*-amyl chlorocarbonate was added dropwise. This solution was warmed to room temperature, stirred for about 1 hr., and cooled to about −30°. Then 2 g. of *n*-amyl chlorocarbonate was added, and the solution was stirred for an additional 2 hr. The pyridine solution was poured into 1 l. of ice water acidified to pH 2 with concentrated hydrochloric acid. The resulting aqueous suspension was extracted with two 250-ml. portions of chloroform, and the chloroform extracts were combined and dried over anhydrous magnesium sulfate. The chloroform was removed under vacuum at 38°. The resulting white precipitate was shaken with 750 ml. of anhydrous ether; the product was collected by filtration, air dried for about 1 hr., and then dried for about 16 hr. under vacuum at 45°. The crude product (6.75 g.) contained both the 3-ester and the 2-ester in about a 2:1 ratio (Fig. 1) and was equilibrated between 700 ml. of ether and 460 ml. of aqueous buffer of pH 2.2. The ether layer was removed and discarded, and the aqueous layer was extracted again with 800 ml. of ether. The aqueous layer was extracted with 780 ml. of chloroform (in two portions), and the chloroform extracts were pooled, dried with anhydrous sodium sulfate, and evaporated to dryness under vacuum at about 50°. The resulting residual light-yellow oil was taken up in 80 ml. of chloroform, which was made acidic with anhydrous hydrogen chloride, and again reduced to dryness under vacuum at about 50° to give 2.65 g. (49%) of white solid, 7(S)-chloro-7-deoxylincomycin 3-pentylcarbonate hydrochloride. The GLC[5] pattern (Fig. 2) showed only a trace of the 2-ester.

Clindamycin 2,3-Dicarbonate Esters [7(S)-Chloro-7-deoxylincomycin 2,3-Dihexylcarbonate Hydrochloride]—7(S)-Chloro-7-deoxylincomycin hydrochloride monohydrate (4.79 g., 0.01 mole) was dissolved in 75 ml. of analytical reagent grade pyridine and cooled to −30°. Hexyl chloroformate[4] (6.59 g., 0.04 mole) was added dropwise with rapid stirring. The reaction mixture was slowly warmed to room temperature and stirred for 2 hr. TLC (silica gel plates, Solvent A) indicated that the reaction was not complete, so an additional 1.5 g. of hexyl chloroformate was added to the recooled (−30°) reaction mixture. The mixture was again warmed to room temperature and stirred for 1 hr. The reaction mixture was again cooled and an additional 0.5 g. of hexyl chloroformate was added. The pyridine solution was poured into 1 l. of ice water and extracted with two 250-ml. portions of ether. The combined ether fractions were dried with anhydrous magnesium sulfate. Ether and pyridine were removed under reduced pressure, and the resulting syrup was dissolved in 100 ml. of anhydrous ether. Anhydrous hydrogen chloride gas was bubbled through the ether solution for 2 min. After standing for 10 min., a gelatinous precipitate formed. The ether was removed under reduced pressure (water aspirator), and the resulting tan syrup solidified on standing. The solidified syrup was shaken with two 75-ml. portions of anhydrous ether and filtered.

Clindamycin Salts—*7(S)-Chloro-7-deoxylincomycin Lactobionate*—Lactobiono-δ-lactone (13.61 g., 0.04 mole) was dissolved in 100 ml. of water, heated on a steam bath for 1 hr., and filtered. This solution was added slowly to 17 g. (0.04 mole) of 7(S)-chloro-7-deoxylincomycin base dissolved in 50 ml. of acetone. The resulting yellow solution was stirred for 1 hr., and then all solvent was removed *in vacuo* (200 μ) at 38°. The resulting syrupy precipitate was shaken with 100 ml. of anhydrous acetone for 1.5 hr. The resulting gum was rubbed in a mortar with a small amount of acetone until a homogeneous suspension was formed. The suspension was filtered, and the precipitate was dried *in vacuo* for 1 hr. at 55°.

7(S)-Chloro-7-deoxylincomycin Gluconate—δ-Gluconolactone (7.1 g., 0.04 mole) was dissolved in 50 ml. of distilled water, heated on a steam bath for 1.5 hr., and then cooled to room temperature. This solution was added dropwise to 17 g. (0.04 mole) of 7(S)-chloro-7-deoxylincomycin base dissolved in 25 ml. of anhydrous acetone. The resulting solution was stirred at room temperature for 1 hr. The solvent was removed *in vacuo*, and the resulting gum was treated with acetone. About 20 g. (80%) of white amorphous powder was obtained and dried *in vacuo*.

[5] Instrument, F&M 700 with flame-ionization detector; carrier gas, helium (20 ml./min.); column, 43.2 × 1.3-cm. (17 × 0.5-in.) stainless steel; support, Gas Chrom Q (100–200 mesh); stationary phase, 1% OV-1; injector port temperature, 275°; detector temperature, 275°; oven temperature, linearly programmed from 200 to 245° at 3°/min.; chart speed, 0.5 in./min.; and attenuation, $10^2 \times 5$.

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