Synthesis and Bioactivity of Lincomycin-2-Phosphate

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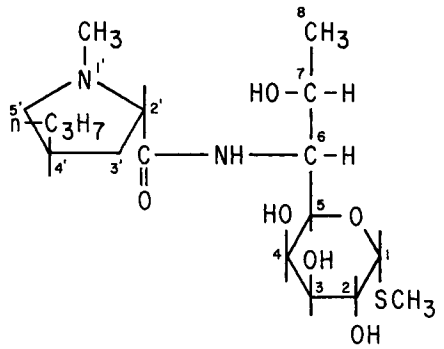
Abstract □ Tritylation of 3,4-*O*-anisylidene lincomycin (II) was found to give 7-*O*-trityl-3,4-*O*-anisylidene lincomycin (III), a key intermediate in the synthesis of lincomycin-2-monoesters. The structure of III was established by NMR. Phosphorylation of III with $POCl_3$ in pyridine gave the resulting dichlorophosphate IV which after hydrolysis and treatment with 80% aqueous acetic acid (100°, 30 min.) gave lincomycin-2-phosphate (V). The water-soluble ester V is inactive in the plate antibacterial assay using *S. lutea*, although *in vivo* V is as active as the parent compound lincomycin (I) in mice infected with *S. aureus*. V gave slightly higher blood levels than the parent compound I upon oral administration in dogs. The taste of V is less bitter than I.

Keyphrases □ Lincomycin-2-phosphate—synthesis □ Bioactivity—lincomycin-2-PO_4 □ Ion exchange chromatography—separation □ TLC—separation, identification □ NMR spectroscopy—structure



I
Ia = HCl salt

This work is part of a program aimed at improving the pharmaceutical (taste, *etc.*) and biological properties (absorption, depot action, *etc.*) of the antibiotic lincomycin (I) (1–3). The problem can be approached by (*a*) the synthesis of esters which could be cleaved by *in vivo* enzyme systems, or (*b*) the synthesis of esters which are labile at physiological pH. This report describes the synthesis of the enzymatically labile phosphate ester of lincomycin. The synthesis of hydrolytically labile esters will be reported later.

The excellent chemical stability of phosphate esters (4) in the di-anionic state (>pH 6) and the ease of *in vivo* hydrolysis by the widely distributed phosphatases makes this class of derivatives ideal for modifying the physical properties of drugs while maintaining the *in vivo* bioactivity of the parent compound.

It was speculated that lincomycin-2-phosphate (V) might have a less bitter taste than I because (*a*) imparting a net negative charge on the originally positively charged I might disturb or prevent the bitter taste receptor interaction and (*b*) heavy metal or amine salts might be insoluble and therefore less bitter.

This report shows that V is antibacterially active *in vivo* and that it is less bitter than I.

RESULTS AND DISCUSSION

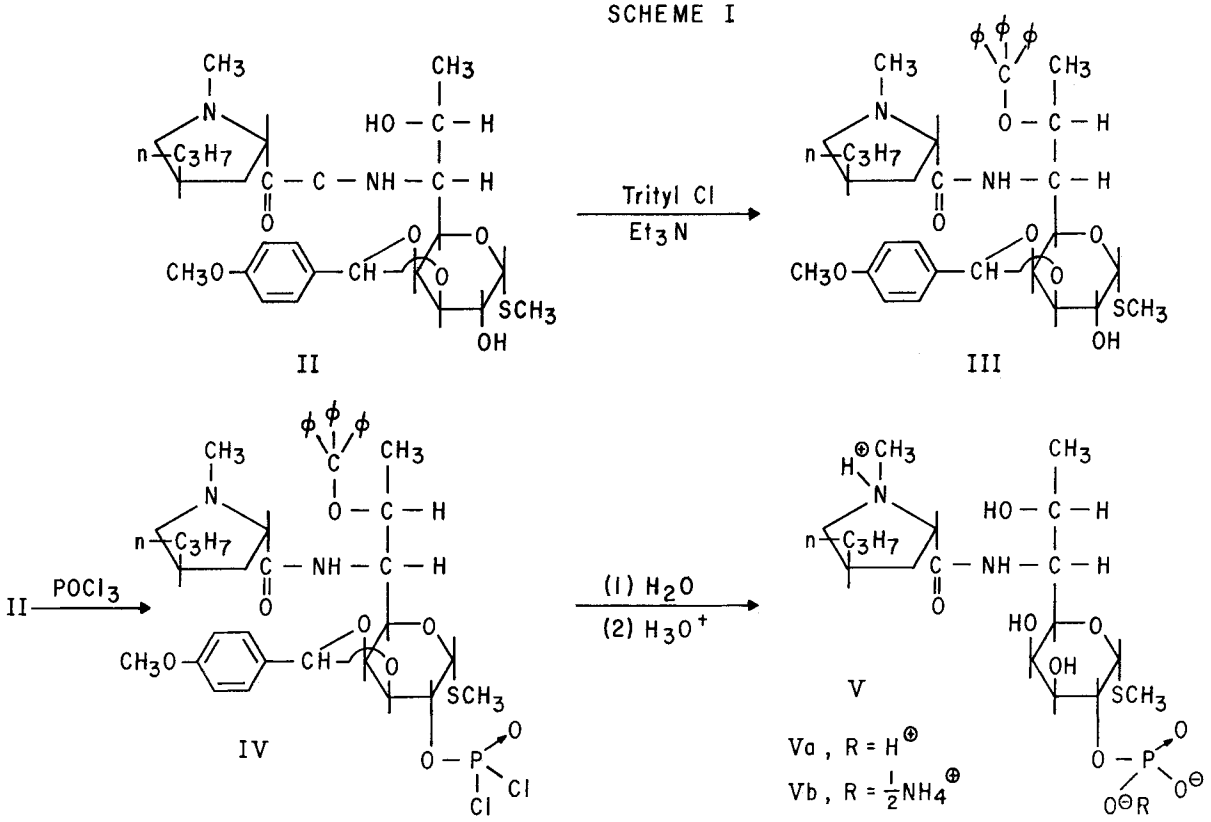
Synthesis—Lincomycin-2-phosphate (V) was synthesized by the route shown in Scheme I. Reaction of 3,4-*O*-anisylidene lincomycin (II) (5) with trityl chloride and triethylamine in acetone provided selective tritylation at the 7-position to give 7-*O*-trityl-3,4-*O*-anisylidene lincomycin (III). To establish the structure, 7-*O*-trityl-3,4-*O*-anisylidene lincomycin-2-benzoate (VI) was prepared from III with benzoyl chloride in chloroform-pyridine. Subsequent removal of the trityl and anisylidene groups with 80% aqueous acetic acid afforded lincomycin-2-benzoate isolated as its hydrochloride salt (VII). The structure of III was concluded from its NMR spectrum and the spectra of the resulting benzoate esters (VI and VII) (see Table I).

The NMR spectra show that on tritylation the C_8 methyl doublet in III is shifted upfield by 0.37 p.p.m., the anomeric hydrogen absorption is at the same chemical shift as in I and II, the compound contains one hydroxyl group, and one carbinol hydrogen is shifted upfield due to the trityl ether function. The NMR spectrum of the benzoate ester VI retains the C_8 methyl doublet shifted upfield and the trityl ether carbinol hydrogen is shifted upfield. The spectrum contains aromatic hydrogen absorption characteristic of a benzoate ester and a complex pattern at 5.5 δ with area equivalent to two hydrogens. The line intensities and splitting patterns of this complex multiplet indicate that the two hydrogens are on adjacent carbons, the anomeric hydrogen is shifted downfield and its usual doublet pattern is distorted by the proximity of the C_2 hydrogen absorbance. Acid-catalyzed removal of the trityl and anisylidene protecting groups of VI gave the monobenzoate VII. The NMR spectrum of VII retained the 5.6–5.4 δ absorption complexities of

Table I—NMR Spectral Data, δ Scale

Compound	Solvent	Anisylidene: No. Aryl-H	Anisylidene: OCH_3	Anisylidene: Benzylic-H	H_1	$J_{1,2}$, c.p.s.	Carbinol	N—CH_3	C_8-doublet
I	$CDCl_3$	—	—	—	5.35	5.0	4.4–3.4	2.40	1.20
	D_2O	—	—	—	5.34	5.5	4.3–3.4	2.33	1.14
Ia	D_2O	—	—	—	5.30	5.5	4.6–3.5	2.98	1.17
II	$CDCl_3$	4	3.80	6.16	5.31	5.5	4.62–3.8	2.27	1.20
III	$CDCl_3$	19	3.79	6.01	5.22	4.5	4.7–3.65	2.27	0.83
VI	$CDCl_3$	24	3.82	6.07	~5.52	~5.5	H_2 ~5.48, 4.85–3.65	2.27	0.95
VII	d_7DMF	5 AR-H's of benzoate			5.63	5.5	H_2 ~5.45, 4.7–3.5	3.04	1.21
V	D_2O	—	—	—	5.53	5.5	4.7–3.65	2.95	1.16

SCHEME I



the anomeric and C_2 hydrogens, whereas the C_8 methyl doublet shifted back to the position of the corresponding functions in I and II. Spin decoupling techniques at 100 Mc.p.s. demonstrate that esters prepared from III exhibit esterified carbinol hydrogen absorption in the 5.0–5.4 δ region with the 5.5 c.p.s. vicinal coupling of the anomeric hydrogen. One concludes that the esters VI and VIII are C_2 esters and therefore the trityl function in III must be at C_7.

Treatment of III with $POCl_3$ in pyridine gave the dichlorophosphate intermediate IV, which was hydrolyzed to the ionic phosphate ester on treatment with water. The trityl and anisylidene protective groups were removed with 80% aqueous acetic acid at 100° in 15–30 min. The phosphate ester V was initially isolated by preparative ion-exchange chromatography on ion-exchange resin[1] using a linear gradient of water (pH 9) going to 1 *N* ammonium acetate (pH 9).

The chromatographic conditions were dictated by the results of sorption studies. A pH of 9 was chosen because V should have an effective charge of −2 at high pH (Fig. 1), assuming a pKa of 7.5 for the amino group[2] and pKa values of 2 and 6 for the phosphoryl group (6). V was efficiently sorbed by the cationic resin[1] at pH 8–9 (Fig. 2). Complete desorption of V from the resin occurred with approximately 0.3 *N* ammonium acetate at pH 9 (Fig. 3).

In the preparative chromatographic system (Fig. 4), a linear gradient of water (pH 9) to 1 *N* ammonium acetate was used. This system permits sorption of V at low electrolyte concentration and complete desorption of V before reaching the maximum electrolyte concentration. The phosphate ester peak (Fig. 4, Peak II) was collected from the preparative chromatographic system and the solution was freeze-dried to remove ammonium acetate. Recrystallization of the freeze-dried cake gave the hemiammonium salt V*b* in 26% yield.

The homogeneity of the compound was established by the presence of one symmetrical peak in the analytical ion-exchange chromatogram (Fig. 5) with only a trace of a supposed pyrophosphate ester of I. Only one signal was observed for the thiomethyl, *N*-methyl, and the anomeric proton doublet in the 60 Mc.p.s. NMR spectrum (see Table I).

The demand for kilogram quantities of V necessitated a process development study. This study afforded a phosphorylation procedure which permitted purification of V by direct crystallization of the zwitterionic form V*a* from alcohol–water mixtures. The main changes in the improved procedure were the use of large excess of $POCl_3$ and the removal of inorganic phosphate from the fully protected phosphate ester. Yields of approximately 50% were obtained on the kilogram scale with a purity of greater than 99% as shown by analytical ion-exchange chromatography.

Bioactivity—Three procedures were used to compare the biological activities of V*a*, V*b*, and the parent compound, I*a*. Activities were compared in a microbiological assay using *S. lutea* (7), therapeutic activities were compared following oral and subcutaneous administration to *S. aureus*-infected mice (7), and fasting blood levels were compared after oral administration of 0.123 mmoles/kg. to the three beagle dogs.

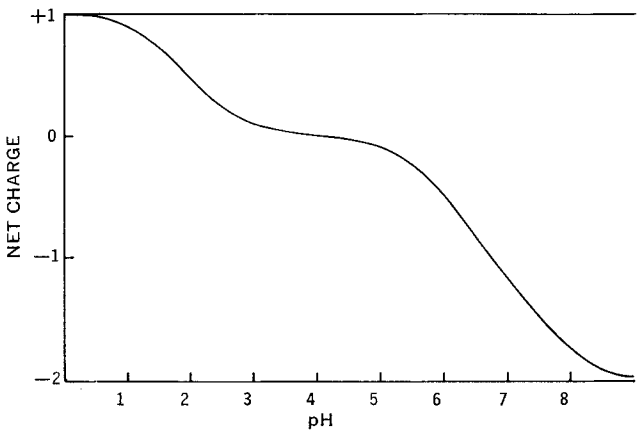


Figure 1—*Calculated net charge-pH profile for lincomycin-2-phosphate (V) using the pKa assignments of 2 and 6 for the phosphoryl moiety and 7.5 for the amino function.*

[1] Dowex 1-X2, The Dow Chemical Co., Midland, Mich.
[2] The pKa of lincomycin·HCl is 7.5 in water.

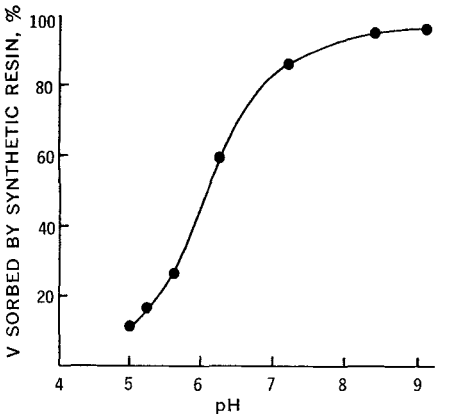


Figure 2—*Percentage of lincomycin-2-phosphate (V) sorbed* versus *pH during continuous recirculation of 50 ml. water containing 0.2% V through 1 g. ion-exchange resin (acetate form, 200–300 mesh). The pH was changed by addition of gaseous ammonia.*

Compounds V*a* and V*b* had identical activities using either the therapeutic or microbiological assay. Although I*a* was more than 50 times as active as V*a* in microbiological assays the two compounds had equal therapeutic activities in *S. aureus*-infected mice, whether administered orally or subcutaneously. In blood level studies (Fig. 6), V*a* blood levels were slightly higher than those obtained with the parent compound. Both I*a* and V*a* gave peak blood levels 1 hr. after oral administration.

These studies indicate that antibacterial activity probably depends on lincomycin levels, that the unhydrolyzed ester is inactive, and that *in vivo* hydrolysis of the phosphate ester V*a* to the parent compound is very rapid. Hydrolysis of the phosphate ester by the widely distributed phosphatases probably accounts for the *in vivo* activity of V*a*.

Taste Panel Evaluation—Aqueous formulations of V and I*a* (0.123 *M*) were prepared containing 65% sucrose, 0.385% saccharin, preservatives, imitation raspberry flavor, and coloring agents.

The formulations were coded and submitted for taste evaluation to a panel of 36 adults. The taste was rated on a scale of 1 (very bitter) to 9 (tasteless). The results showed an average score of 4.68 for V and 3.39 for I*a*. The difference in means required for significance at the 5% level was 0.86, thus indicating that V is statistically less bitter than I*a*.

Various long chain amine salts of V and the poorly water soluble calcium salt of V failed to improve the taste.

EXPERIMENTAL

Thin-Layer Chromatography (TLC)—TLC was conducted on cellulose (Avicel) and on Silica Gel G plates. Solvent A: hexane, ether, pentan-2-one, methanol, concentrated ammonium hydroxide

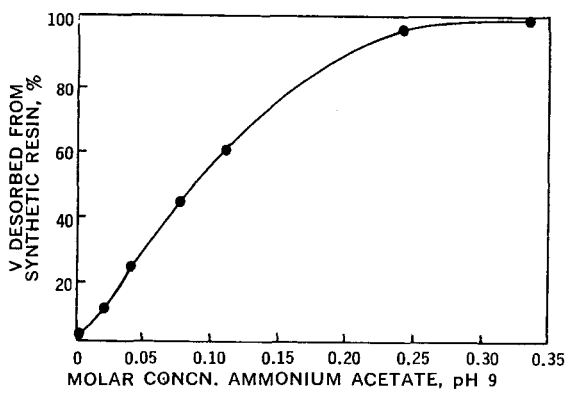


Figure 3—*Percentage lincomycin-2-phosphate (V) desorbed from ion-exchange resin by ammonium acetate at pH 9.0 using the circulating device and quantities as in Fig. 2.*

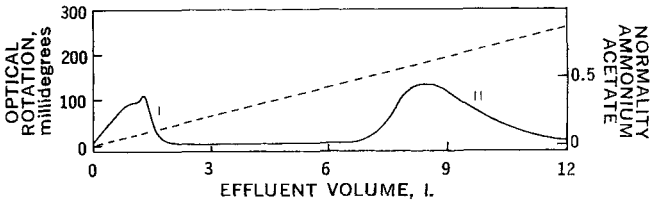


Figure 4—*Preparative ion-exchange chromatogram of 40 g. lincomycin-2-phosphate (V) on a 7.62-cm. × 35.56-cm. (3-in. × 14-in.) column of ion-exchange resin (200–400 mesh, acetate form) using a linear gradient (dashed line) consisting of 7 l. water (pH 9) going to 7 l. 1 N ammonium acetate (pH 9). The column effluent (flow rate 1.5 l./hr.) was monitored continuously with a Bendix recording polarimeter. Peak I is mainly the starting Compound I and Peak II is the phosphate ester V.*

(60:20:20:9:1). Solvent B: *n*-butanol, isopropyl alcohol, water, concentrated ammonium hydroxide (10:70:20:10). The silica gel plates were sprayed with 20% ammonium sulfate and heated to 180° to char the compounds. Iodine was used to detect the compounds on cellulose plates.

NMR Spectra at 60 Mc.p.s.—Chemical shifts are reported in delta (δ) units, *i.e.*, p.p.m. downfield from the internal reference. Samples in $CDCl_3$ and d_7 DMF (~0.0–0.15 *M*) utilize the internal standard tetramethylsilane. Samples in D_2O (~0.1–0.15 *M*) utilize sodium2,2-dimethyl-2-sila-pentane-5-sulfonate as internal reference. Hydroxyl resonance peaks were verified *via* exchange reactions with D_2O. Table I lists the pertinent NMR data.

3,4-*O*-Anisylidene Lincomycin (II)—II was prepared by a modification of the procedure reported earlier (5). Three hundred grams of anhydrous lincomycin HCl was dissolved in a mixture of 1600 ml. *N*,*N*-dimethylformamide and 500 ml. anisaldehyde by heating to 94°. The solution was diluted with 1175 ml. benzene and the solvent was allowed to distill. After collecting 400 ml. of distillate, 400 ml. of fresh benzene was added. This process was continued until 5.2 l. of distillate was collected. Crystallization of the hydrochloride salt of II occurred during distillation. After standing at RT for 18 hr., the pale yellow crystalline compound was isolated by filtration under a nitrogen atmosphere. The filter cake was washed with chloroform and the moist cake was dissolved in a mixture of 6 l. methylene chloride and 10 l. of water containing 35 g. sodium hydroxide. The methylene chloride layer was washed successively with 1 l. water, 100 ml. 0.1 *N* HCl and finally with 1 l. water. The organic layer was concentrated to 500 ml. by atmospheric distillation and then concentrated to a viscous residue under high vacuum. The residue was dissolved in 450 ml. hot acetone and the solution was decolorized with 15 g. charcoal. After filtration, the solution was diluted with 1800 ml. ether followed by 820 ml. hexane. The resulting crystals (273 g., 77%) were isolated by filtration and dried at 65° under high vacuum, m.p. 136–137°. TLC on silica gel showed one spot in Solvent A with R_f 0.3.

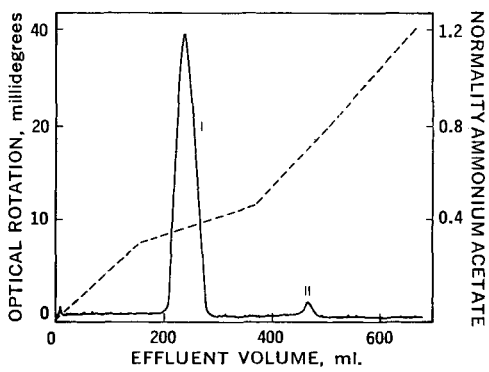


Figure 5—*Analytical ion-exchange analysis of lincomycin-2-phosphate V*b *(35.6 mg.) on a 9- × 400-mm. column of ion-exchange resin (200–400 mesh, acetate form) using three linear gradients going from water (pH 9) to 1.2 N ammonium acetate (pH 9). The eluant was pumped at a rate of 2 ml./min. and the effluent was monitored continuously using a Bendix recording polarimeter. Peak I is the phosphate ester V; Peak II is probably the pyrophosphate ester of I.*

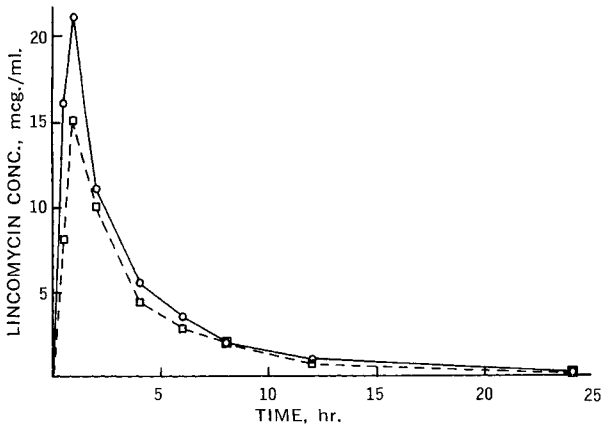


Figure 6—*Average serum levels in three dogs following oral administration of lincomycin-2-phosphate (Va) (○—○) and lincomycin·HCl (Ia) (□—□) both at a dosage equivalent to 50 mg./kg. of lincomycin base (0.123 mM/kg.).*

Anal.—Calcd. for $C_{26}H_{40}N_2O_7S$: C, 59.53; H, 7.69; N, 5.34; S, 6.10; eq. wt., 524.63. Found: C, 59.58; H, 7.94; N, 5.75; S, 6.21; eq. wt. 561; $[\alpha]_D$ + 137° (EtOH).

7-*O*-Trityl-3,4-*O*-Anisylidene Lincomycin (III)—A solution containing 128 g. of 3,4-*O*-anisylidene lincomycin in 400 ml. acetone was treated with 256 ml. triethylamine and 320 g. trityl chloride. The condenser was fitted with a $CaCl_2$ drying tube and the suspension was refluxed for 24 hr. with concomitant precipitation of triethylamine HCl.

The brown suspension was cooled to 50° and 125 g. silica gel was slowly added. The mixture was stirred for about 2 min. and then subjected to filtration using a heated filter funnel. The solids were washed with two 200-ml. portions of hot acetone. The brown filtrate and washings were combined and diluted with 1600 ml. cyclohexane. The solution was heated to reflux while adding 5600 ml. hexane and after standing at RT overnight the resulting yellow crystals were isolated by filtration, washed with 500 ml. hexane, and air-dried to give 160 g. (86%) of crude III.

The product was dissolved in 7400 ml. hot acetone and the solution was quickly filtered through a bed of 800 g. silica gel. The silica gel bed was washed with 4500 ml. hot acetone and the filtrate and washings were combined.

The colorless solution was heated to reflux and then diluted with 12 l. of 55° water. Crystallization of the product occurred while cooling the solution to 10°. The crystals were isolated by filtration, washed with acetone-water (2:1), and then air-dried to give 135 g. (72%) of III, m.p. 202–203°. TLC on silica gel in Solvent A showed one spot with R_f 0.6.

Anal.—Calcd. for $C_{45}H_{54}N_2O_7S$ (767.01): C, 70.47; H, 7.10; N, 3.65; S, 4.18. Found: C, 70.66; H, 7.19; N, 3.93; S, 4.33.

7-*O*-Trityl-3,4-*O*-Anisylidene Lincomycin-2-Benzoate (VI)—One gram of III was dissolved in a mixture of 10 ml. chloroform and 1.1 ml. pyridine in a flask protected from moisture. A solution of 0.60 ml. of benzoyl chloride in 1 ml. chloroform was added dropwise over a 0.5-hr. period.

After dilution with 25 ml. $CHCl_3$, the solution was extracted with 200 ml. 5% Na_2CO_3 and then extracted with three 200-ml. portions of water. The chloroform layer was dried with sodium sulfate and about 10% of the chloroform was removed by distillation. The solution was diluted with 40 ml. hexane and gentle scratching of the flask induced crystallization. The compound was isolated by filtration and air-dried to give 805 mg. of VI. Slow evaporation of the combined filtrates gave an additional 95 mg. of product (combined yield 80%), m.p. 144–145°.

Anal.—Calcd. for $C_{52}H_{58}N_2O_8S$ (871.12): C, 71.70; H, 6.71; N, 3.22; S, 3.68. Found: C, 71.32; H, 6.89; N, 3.42; S, 3.71.

Lincomycin-2-Benzoate HCl (VII)—A solution of 490 mg. of VI was dissolved in 4 ml. glacial acetic acid. The solution was diluted with 0.8 ml. water and then heated at 100° for 20 min. The hot solution was diluted with 5 ml. water resulting in crystallization of tritanol. After cooling, the tritanol was removed by filtration and the filtrate was concentrated to a viscous residue under vacuum at 25°. The residue was dissolved in 25 ml. water and the solution was treated with 10 ml. 3% sodium carbonate. The resulting white precipitate of lincomycin-2-benzoate was extracted with four 15-ml. portions of chloroform. The extracts were combined, dried with sodium sulfate, and concentrated to about 12 ml. by atmospheric distillation. The solution was evaporated to a viscous residue under vacuum at 25° and the residue was dissolved in 35 ml. ether. The solution was cooled to 0°, HCl gas was introduced, and the resulting precipitate was isolated by filtration under nitrogen. The product was washed with ether and dried under vacuum to give 270 mg. of white amorphous product.

The compound (240 mg.) was dissolved in 6 ml. tetrahydrofuran, treated with 50 mg. charcoal, and filtered. The filtrate was diluted with 10 ml. acetone and then slowly diluted with 20 ml. ether over a 5-min. period to precipitate an amorphous powder. The compound was isolated by filtration under nitrogen and dried under vacuum to give 225 mg. (81%).

Anal.—Calcd. for $C_{25}H_{39}ClN_2O_7S$ (547.13): C, 54.88; H, 7.19; Cl, 6.48; S, 5.86. Found (corrected for H_2O): C, 55.59; H, 7.58; Cl, 6.43; S, 5.83; H_2O, 4.40.

Hemi-ammonium Lincomycin-2-Phosphate (Vb)—A solution of 76.7 g. of III in 200 ml. pyridine was added dropwise over a period of 10 min. to a stirred solution of 18.4 g. $POCl_3$ in 200 ml. pyridine maintained at −40°. The solution was allowed to warm to −20° and after 25 min. the solution was mixed with a cold (−35°) mixture of 36 ml. water and 150 ml. pyridine. The solvent was removed at 55° under vacuum. Ethanol (100 ml.) was added to the viscous residue and the solvent was removed again. The residue was dissolved in 400 ml. acetic acid, the solution was diluted with 80 ml. water and heated on a steam bath for 0.5 hr. The solvent was removed under vacuum at 55°, 200 ml. water was added, and the evaporation process was repeated. The residue was shaken with a mixture of 700 ml. water and 100 ml. concentrated ammonium hydroxide. The insoluble tritanol was removed by extraction with 1 l. chloroform. Ammonia was removed under vacuum and the solution was freeze-dried to give 75 g. crude V.

A solution of 40 g. crude V in 750 ml. water was adjusted to pH 9 with NH_4OH and then applied to a 7.62 × 35.56-cm. (3-in. × 14-in.) column of resin (acetate) prewashed with 0.1% NH_4OH. The rate of application was 750 ml./hr. Elution was conducted at a rate of 1500 ml./hr. with a linear gradient of 7 l. water (pH 9) going to 7 l. 1 *N* ammonium acetate (pH 9). The column effluent was monitored continuously with a Bendix recording polarimeter (Fig. 4). The product peak was collected separately and concentrated to a low volume to remove most of the ammonium acetate. The solution was diluted with 4 l. water and freeze-dried. The residual cake was heated to 100° under high vacuum to remove traces of ammonium acetate. The only contaminant at this stage was inorganic phosphate which was removed with ammonia. A solution of the compound in a mixture of 200 ml. water and 200 ml. ethanol was cooled to 0° and saturated with gaseous NH_3 to precipitate ammonium phosphate. The precipitate was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in a mixture of 66 ml. water and 3.6 ml. acetic acid. The solution was diluted with 450 ml. acetone to induce crystallization. The product was isolated, dried at 100° under high vacuum, and equilibrated with the laboratory atmosphere to give 14.2 g. (26%) of the hemi-ammonium salt V*b*, m.p. 201–208°.

The compound showed one spot (R_f 0.5, Solvent B) on cellulose TLC and only a trace of a supposed pyrophosphate impurity was observed in the analytical ion-exchange chromatogram (Fig. 5).

The elemental analyses indicated the compound was the hemi-ammonium salt V*b*. The discrepancies in the analyses were probably due to the unstable character of the ammonium salt.

Anal.—Calcd. for $C_{36}H_{73}N_5O_{18}P_2S_2$ (990.10). C, 43.67; H, 7.43; N, 7.07; P, 6.26; eq. wt., 245.02. Found: C, 42.22; H, 7.90; N, 7.07; P, 5.77; eq. wt., 237.; H_2O, 6.91 (analyses corrected for 6.91% H_2O and 0.87% inorganic phosphate as $NH_4H_2PO_4$).

Lincomycin-2-Phosphate (Va)—Preparative Scale—A solution of 300 g. III in 2600 ml. pyridine was added dropwise to a stirred solution of 125 g. $POCl_3$ in 850 ml. pyridine maintained at −5°. After 45 min. at −5° the solution was diluted with a cold (0°) mixture of 800 ml. pyridine, 1600 ml. tetrahydrofuran, and 700 ml. water. The resulting suspension was concentrated to 1500 ml. under vacuum at 40° and 13 l. water was added. The product was isolated by filtration and then dissolved in 3 l. hot (85°) 80% aqueous acetic acid. After 30 min. at 85°, the solution was concentrated under vacuum at 60° to 1600 ml. resulting in precipitation of tritanol. Two hundred milliliters water was added and the tritanol was re-

moved by filtration. The filtrate was concentrated to 600 ml. under vacuum at 60° and 6 l. acetone was added resulting in crystallization of crude V*a*. The compound was dissolved in 1 l. water and extracted with 300 ml. chloroform. The pale yellow, aqueous layer was partially decolorized with charcoal and the solution was concentrated to 300 ml. under vacuum at 50°. The solution was diluted with 400 ml. ethanol and the resulting white crystalline V*a* was isolated by filtration. The compound was recrystallized from a mixture of 500 ml. hot (80°) water by addition of 500 ml. ethanol. The product was isolated by filtration giving 95 g. (50%), m.p. 223–225° (dec.). Cellulose TLC (Solvent B) and analytical ion-exchange chromatography showed no impurities.

Anal.—Calcd. for $C_{18}H_{35}N_2O_9SP$ (486.52): C, 44.43; H, 7.25; N, 5.76; P, 6.37; S, 6.59; eq. wt., 486.52. Found: C, 44.70; H, 7.40; N, 5.90; P, 6.50; S, 6.60; eq. wt., 492.; H_2O, 3.15 (analyses corrected for 3.15% H_2O); $[\alpha]_D^{29}$ + 120° (H_2O).

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Determination of Phenethanolamine Drugs in Biologic Specimens by Ultraviolet Spectrophotometry

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Abstract □ A rapid differential method for spectrophotometrically determining ephedrine and certain related compounds in biologic specimens in the presence of other alkaline drugs is presented. In the procedure, the compounds are oxidized by means of alkaline periodate to benzaldehyde which, in comparison to the original compounds, has a much higher molar absorptivity for UV radiations. In order to enhance significantly the sensitivity and specificity of the procedure benzaldehyde is subsequently converted to its semicarbazone derivative. Only compounds that have a benzyl alcohol functional group and are extractable as an alkline drug can be analyzed by the method. The procedure is sufficiently sensitive to permit analysis of the compounds in biologic specimens after therapeutic doses.

Keyphrases □ Phenethanolamine in biological specimens—determination □ Periodate oxidation—phenethanolamine determination □ GLC—identification □ IR spectrophotometry—identification □ UV spectrophotometry—analysis

The extensive use of phenethanolamine compounds in pharmaceutical preparations has led many investigators to develop rapid and sensitive procedures for determining these compounds in biologic and pharmaceutical specimens. The many published methods attest not only to the importance of the drugs but also to the difficulties inherent in their analysis. Ephedrine (1-phenyl-2-methylamino-propanol) and the related compounds phenyramidol, pseudoephedrine, and phenylpropanolamine have benzenoid spectra which are neither sensitive nor characteristic in their absorption of UV radiations. Methods which rely on direct UV techniques, therefore, have little application to the analysis of these compounds in biologic systems (1, 2).

The colorimetric procedures (3–5) for determining ephedrine-type compounds also give positive reactions with many other alkaline-extractable drugs. The fluorometric methods (6) detect submicrogram quantities but require specialized instrumentation and are often time-consuming. The problems in applying gravimetric or nonaqueous titration systems (7) to the analysis of compounds in biologic specimens are evident. Paper (8) and gas chromatographic (9, 10) procedures can be made very sensitive, but they do not provide the forensic scientist with specific information concerning the molecular structure of the unknown compounds. None of the previously mentioned methods are capable of screening large numbers of samples in a short time period which is often necessary in the clinical laboratory.

Using periodate oxidation, Heimlich (11) developed a specific analytical technique for determining phenylpropanolamine in urine. Derivative formation by his method affords a sensitivity three to four times greater than that for direct spectrophotometric determination of unconverted phenylpropanolamine. Periodate oxidation was also used by Chafetz (12) for the assay of several phenethanolamine compounds in pharmaceutical preparations, but the procedure is not applicable to the analysis of the drugs in biologic specimens.

The use of steam distillation and a dichromate reagent to obtain benzaldehyde from ephedrine was reported