

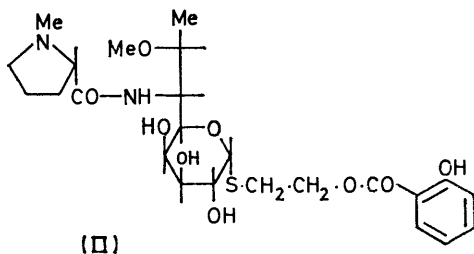
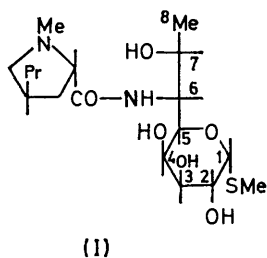
## Modifications of Lincomycin Involving the Carbohydrate Portion. Part III.<sup>1</sup> The 7-*O*-Methyl and 6-De-(1-hydroxyethyl) Analogues

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7-*O*-Methyl-lincomycin has been synthesised, by use as intermediates of the carbohydrate portions of both the antibiotics lincomycin and celesticetin. The use of the latter involved the development of a method for introducing the axial glycosidic methylthio-substituent, and this method permitted the synthesis of 6-de-(1-hydroxyethyl)-lincomycin from *D*-galactose. The 7-*O*-methyl analogue possesses enhanced antibacterial activity, whereas the analogue lacking C-7 and C-8 shows 1% of the antibacterial activity of the parent in *in vitro* tests.

MODIFICATION in the antibiotic lincomycin † (I) of the 2-substituent,<sup>2</sup> and the stereochemistry at positions 1–4<sup>1,2</sup> in the carbohydrate ring, results in drastic loss of antibacterial activity in *in vitro* tests. However, modification of the 7-hydroxy-group, both of substituent and of stereochemistry, may result either in only partial loss of activity or in enhancement of both potency and spectrum of activity. Discussion of such modifications is included in a recent review.<sup>3</sup> Further interest in the chemical modification of position 7 stemmed from the similarity in structure between lincomycin and the antibiotic celesticetin<sup>4</sup> (II), in which a methoxy-group is present at C-7 with the same *D*-erythro- $\alpha$ -*D*-galacto-stereochemistry.

Earlier work<sup>5</sup> had made available methyl (7*S*)-*N*-acetyl-7-chloro-7-deoxy-1-thiolineosaminide (III), and the possibility of displacement of the chlorine by methoxide was examined. Since attempted displacement by the weakly basic sodium azide in *NN*-dimethylformamide



had resulted in the formation of the 4,7-anhydro-derivative<sup>6</sup> (IV), the *N*-acetyl compound was converted into its 3,4-acetonide (V), which was heated in methanolic sodium methoxide. The syrupy, chlorine-

† Lincocin is the trademark of The Upjohn Company for lincomycin hydrochloride.

<sup>1</sup> Part II, B. Bannister, *J.C.S. Perkin I*, 1972, 3031.

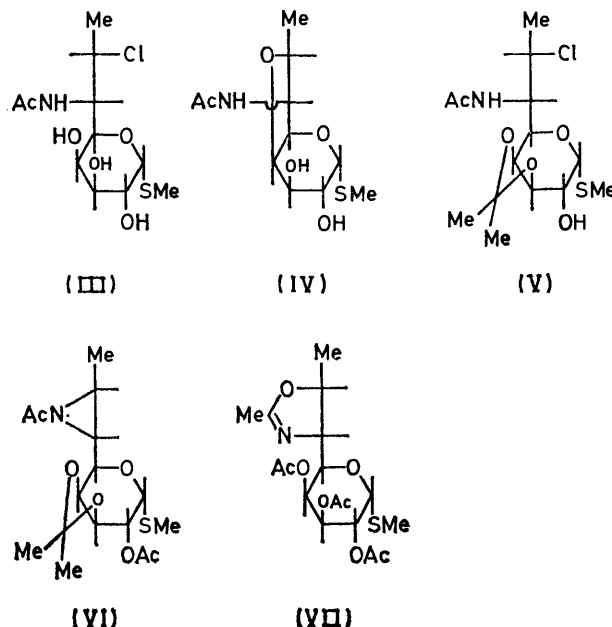
<sup>2</sup> B. Bannister, *J.C.S. Perkin I*, 1972, 3025.

<sup>3</sup> B. J. Magerlein, *Adv. Appl. Microbiol.*, 1971, **14**, 185.

<sup>4</sup> H. Hoeksema, *J. Amer. Chem. Soc.*, 1964, **86**, 4224.

<sup>5</sup> B. J. Magerlein and F. Kagan, *J. Medicin. Chem.*, 1969, **12**, 780.

free product showed no *O*-methyl absorption in the n.m.r. spectrum; its i.r. spectrum showed no amide



II absorption, but an amide I band at 1705  $\text{cm}^{-1}$  indicated that cyclisation to an *N*-acylepimine had occurred.<sup>7,8</sup> The product was characterised as its crystalline 2-*O*-acetyl derivative (VI). Interestingly, the 2,3,4-tri-*O*-acetyl derivative of (III), on treatment with hot  $\gamma$ -collidine, underwent the alternative mode of neighbouring group participation involving the amide carbonyl to give the oxazoline (VII),<sup>9</sup> illustrating the influence of the strength of the base on the course of such reactions when steric requirements are absent.<sup>10</sup>

Since the 7-chloro-compound underwent intramolecular displacement rather than the desired intermolecular displacement in the presence of methoxide anion, attention was turned to the 7-*O*-methylation of methyl thiolineosaminide.<sup>11</sup> The oxazoline acetonide (VIII) has been described as an intermediate in the synthesis of 2-*O*-methyl-lincomycin,<sup>2</sup> and the selective hydrolysis

<sup>6</sup> B. Bannister, unpublished results.

<sup>7</sup> H. L. Spell, *Analyt. Chem.*, 1967, **39**, 185.

<sup>8</sup> H. Saeki, T. Iwashige, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 188; H. Saeki and E. Ohki, *ibid.*, pp. 2471, 2477.

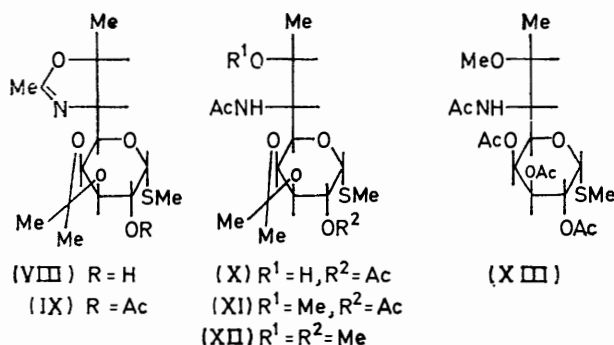
<sup>9</sup> Dr. B. J. Magerlein, The Upjohn Company, personal communication.

<sup>10</sup> J. S. Brimacombe, *Fortschr. Chem. Forsch.*, 1970, **14**, 367.

<sup>11</sup> W. Schoeder, B. Bannister, and H. Hoeksema, *J. Amer. Chem. Soc.*, 1967, **89**, 2448.

of the oxazoline ring by water, without hydrolysis of the acetonide, has been demonstrated.<sup>2</sup> Acetylation of (VIII) in pyridine-acetic anhydride gave a syrupy 2-acetate (IX), hydrolysed in hot water to the crystalline 7-hydroxy-2-acetate (X). Methylation with methyl iodide in the presence of silver oxide gave the 7-*O*-methyl-2-acetate (XI), together with a preponderance of the 2,7-di-*O*-methyl acetonide (XII), identified by comparison with an authentic sample.<sup>2</sup> Hydrolysis of the monomethyl acetonide (XI) in dilute hydrochloric acid, followed by acetylation in pyridine-acetic anhydride, gave the 7-*O*-methyl tetra-acetate (XIII).

The inadequacy of the 2-acetate as a protecting group during methylation led to a consideration of the utilisation of 2-hydroxyethyl thiocelstosaminide (XIV),<sup>4</sup> derived from the antibiotic celesticetin (II), in which the 7-*O*-methyl group already is present. The problem then became one of replacing the 2-hydroxyethylthio-group at C-1 by the required axial methylthio-substituent.



Unlike acid-catalysed glycosidation, the analogous reaction of sugars with alkanethiols produces mainly the acyclic dialkyl dithioacetals and, generally, is unsatisfactory as a method of preparation of thiopyranosides.<sup>12</sup> An alkylthio-substituent at C-1 can be introduced readily from a fully acylated glycopyranosyl bromide. In such solvents as acetone, both the  $\alpha$ - and  $\beta$ -anomeric bromides give the same product, in which the alkylthio-group is *trans* to the ester at C-2. In the case of the  $\beta$ -bromide, the attack of the nucleophile at C-1 is restricted to the  $\beta$ -face of the intermediate 1,2-*cis*-acyloxonium salt resulting from neighbouring group participation.<sup>2,11,12</sup>

Acetylation of 2-hydroxyethyl thiocelstosaminide (XIV) in acetic anhydride-pyridine gave the crystalline *N*-acetyl-tetra-*O*-acetate (XV), which was converted into the acetobromo-derivative (XVI) by reaction with bromine in chloroform.<sup>2,11,12</sup> Introduction of the methylthio-substituent by the method of Cerny and Pacak<sup>13</sup> in acetone solution, *via* the isothiuronium salt, gave the  $\beta$ -thioglycoside (XVII) in high yield.

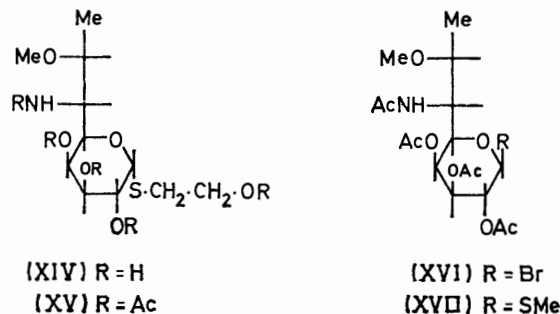
A fully *O*-acylated alkyl  $\beta$ -D-glycopyranoside is converted into an equilibrium mixture of anomers, contain-

<sup>12</sup> D. Horton and D. H. Hutson, *Adv. Carbohydrate Chem.*, 1963, **18**, 123.

<sup>13</sup> M. Cerny and J. Pacak, *Coll. Czech. Chem. Comm.*, 1959, **24**, 2566.

<sup>14</sup> E. Pacsu, J. Janson, and B. Lindberg, *Methods Carbohydrate Chem.*, 1963, **2**, 376.

ing a high proportion of the  $\alpha$ -D-glycopyranoside, in an inert solvent in the presence of a Lewis acid.<sup>14</sup> The preponderance of the  $\alpha$ -anomer at equilibrium is considered to be the result of the destabilisation of the



sterically preferred equatorial anomer owing to the electrostatic interaction between the C(1)-O(alkyl) and C(5)-O(5) bonds, making the axial anomer thermodynamically more stable than the equatorial anomer.<sup>15</sup> This anomeric effect, which applies also to the fully acylated D-glycopyranoses and D-glycopyranosyl halides, would be expected to apply to the acylated alkyl 1-thio-D-glycopyranosides, thus enabling the conversion of the  $\beta$ - into the  $\alpha$ -anomer, although the proportion of the  $\alpha$ -anomer at equilibrium might be lower owing to the larger size of the sulphide sulphur atom relative to the ether oxygen atom, and the weaker electrostatic interaction between the C(1)-S(alkyl) and C(5)-O(5) bonds. Neither methyl *N*-acetyl-2,3,4,7-tetra-*O*-acetyl-1-thio- $\beta$ -lincosaminide<sup>11</sup> nor methyl *N*-acetyl-2,3,4-tri-*O*-acetyl-1-thio- $\beta$ -celstosaminide (XVII) in chloroform under reflux in the presence of either titanium tetrachloride or boron trifluoride showed any indication of anomeric interconversion by t.l.c. in solvent systems in which the anomeric pairs are separated, and starting materials were recovered in high yield. Lack of anomeric interconversion of the corresponding  $\alpha$ -anomers under these conditions was also demonstrated.

If, with glycosides, co-ordination of the Lewis acid with the glycosidic oxygen atom is the first step in the equilibration,<sup>16</sup> resulting in the weakening of the C(1)-OR bond, then the failure to effect equilibration with the analogous thioglycosides may be due to the lower basicity of sulphur compared to oxygen,<sup>17</sup> with consequent lack of effective co-ordination with the catalyst. Such lowered basicity relative to oxygen presumably is involved in the greater stability toward acid hydrolysis of thioglycosides than of glycosides although, in addition, the greater acidity of thiols<sup>18</sup> than of the corresponding alcohols would be expected

<sup>15</sup> R. U. Lemieux, in 'Molecular Rearrangements,' ed. P. de Mayo, Interscience, New York, 1964, part II, p. 709; P. L. Durette and D. Horton, *Adv. Carbohydrate Chem. and Biochem.*, 1971, **26**, 49; J. F. Stoddart, 'Stereochemistry of Carbohydrates,' Wiley-Interscience, New York, 1971, p. 81.

<sup>16</sup> R. U. Lemieux, *Adv. Carbohydrate Chem.*, 1954, **9**, 1.

<sup>17</sup> D. S. Tarbell and D. P. Harnish, *Chem. Rev.*, 1951, **49**, 1.

<sup>18</sup> H. Goldwhite, in 'Rodd's Chemistry of Carbon Compounds,' ed. S. Coffey, 2nd. edn., Elsevier, New York, 1965, vol. 1B, ch. 5, p. 76.

to diminish the efficiency of thiols, relative to alcohols, as leaving groups.<sup>19,\*</sup>

The nucleophilicity of an anion can be increased by the use of a dipolar aprotic solvent because of the low affinity of such solvents towards the solvation of the anion.<sup>22</sup> The efficacy of *NN*-dimethylformamide in the promotion of an entirely  $S_N2$  displacement has been demonstrated by Hughes and his co-workers,<sup>23</sup> who obtained exclusively methyl 1-thio- $\alpha$ -D-ribofuranoside from 2,3,4-tri-*O*-benzoyl- $\beta$ -D-ribofuranosyl bromide by displacement with sodium methanethiolate; in the presence of traces of methanol, the co-production of the  $\beta$ -anomer was noted, resulting from competitive neighbouring group participation.

Replacement of the acetone as solvent by *NN*-dimethylformamide in the Cerny-Pacak sequence with the bromide (XV) gave a mixture of a major and a minor product (t.l.c.). Chromatographic separation yielded the  $\alpha$ -thioglycoside (XIII): the major component, of slightly greater polarity, was the  $\beta$ -anomer (XVII), the ratio of anomers being ( $\alpha$  :  $\beta$ ) 1 : 7.4. The least solvating of the known dipolar aprotic solvents is hexamethylphosphoric triamide,<sup>24</sup> which has been used advantageously in nucleophilic displacements.<sup>25</sup> With this solvent, the products were formed in the ratio ( $\alpha$  :  $\beta$ ) of 1 : 1.9. Long and his co-workers recently have provided another example of the dependence of  $S_N2$  displacements or neighbouring group participation mechanisms on both the strength of the nucleophile and the polarity of the solvent in the displacement reactions of 6-*O*-benzoyl-5-*O*-*p*-tolylsulphonyl-D-glucopyranose derivatives.<sup>26</sup>

The literature contains conflicting reports<sup>27,28</sup> of the stereochemistry of the products of the reactions of thioglycosides with halogen, perhaps owing to the possibility of equilibration of the initial products. We found that the bromination of the  $\beta$ -methylthio-compound (XVII), followed by the Cerny-Pacak sequence in hexamethylphosphoric triamide, gave the same anomeric ratio of methyl thioglycosides (XIII) and (XVII) as resulted from the  $\alpha$ -(2-acetoxyethyl)thio-compound (XV). Hence, the acetobromo-derivative (XVI) is formed with inversion from the  $\alpha$ -, but net retention, due to neighbouring group participation, from the  $\beta$ -thioglycoside (Scheme).

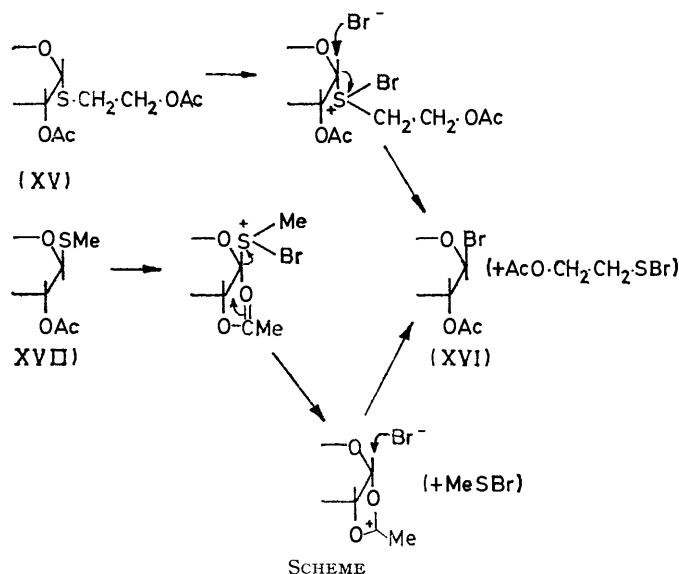
Removal of the protecting acetyl groups of the anomeric tetra-acetyl thioglycosides (XIII) and (XVII)

\* After the completion of this work, reference was found<sup>20</sup> to the conversion of 2-(tetra-*O*-acetyl-1-thio- $\beta$ -D-glucopyranosyl)-4-phenyloxazole and the corresponding thiazole into the  $\alpha$ -anomers in boiling xylene in the presence of mercury(II) bromide. Since the authors record only the yields of the  $\alpha$ -anomers isolated (30 and 5%, respectively) no light is shed on the position of the equilibrium. Pearson's theory<sup>21</sup> of soft and hard acids and bases predicts the greater affinity of the mercury(II) ion as a soft acid for the sulphide sulphur atom as a soft base than of a strong Lewis acid (*e.g.* boron trifluoride) as a hard acid. However, the application of these conditions to the  $\beta$ -anomer (XVII) failed to effect equilibration.

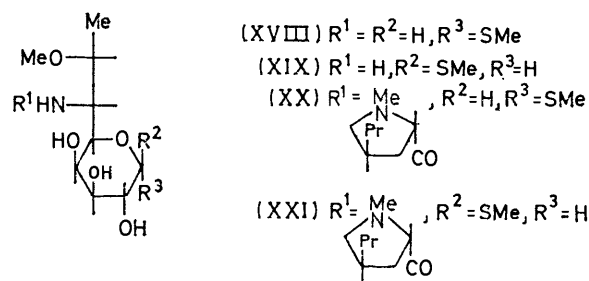
<sup>19</sup> C. Bamford, B. Capon, and W. G. Overend, *J. Chem. Soc.*, 1962, 5138.

<sup>20</sup> P. Nuhn and G. Wagner, *Z. Chem.*, 1967, 7, 154.

by hydrazinolysis<sup>11</sup> gave the crystalline methyl 7-*O*-methyl-1-thio- $\alpha$ - and  $\beta$ -lincosaminides [(XVIII) and (XIX), respectively]. These were converted into their



*N*-1-methyl-*trans*-4-propyl-L-prolyl derivatives by the standard mixed-anhydride procedure,<sup>2,11</sup> and the products were isolated as the crystalline hydrochloride of 7-*O*-methyl-lincosamin (XX) and the amorphous hydrochloride of the  $\beta$ -anomer (XXI).



With the availability of an effective method for introducing the methylthio-substituent axially at C-1 of a pyranose, it became possible to examine the requirement for antibacterial activity in the lincosamin structure of the 1-hydroxyethyl side-chain by the synthesis of the 6-amino-6-deoxygalactose analogue, lacking the C-7 and C-8 groups. An attempt to obtain this analogue by the acid-catalysed cyclisation of

<sup>21</sup> R. G. Pearson and J. Songstad, *J. Amer. Chem. Soc.*, 1967, 89, 1827; R. G. Pearson, *J. Chem. Educ.*, 1968, 45, 581, 643.

<sup>22</sup> A. J. Parker, *Quart. Rev.*, 1962, 16, 163; *Chem. Rev.*, 1969, 69, 1.

<sup>23</sup> C. J. Clayton, N. A. Hughes, and S. A. Saeed, *J. Chem. Soc. (C)*, 1967, 644.

<sup>24</sup> J. J. Delpuech, *Tetrahedron Letters*, 1965, 25, 2111; *Bull. Soc. chim. France*, 1966, 1624.

<sup>25</sup> Y. Ali and A. C. Richardson, *J. Chem. Soc. (C)*, 1968, 1764.

<sup>26</sup> R. C. Chalk, D. H. Ball, and L. Long, jun., *Carbohydrate Res.*, 1971, 20, 151.

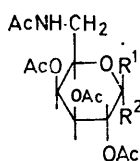
<sup>27</sup> F. Weygand, H. Ziemann, and H. J. Bestmann, *Chem. Ber.*, 1958, 91, 2534; F. Weygand and H. Ziemann, *Annalen*, 1962, 657, 179.

<sup>28</sup> M. L. Wolfrom and W. Groebke, *J. Org. Chem.*, 1963, 28, 2986.

6-deoxy-6-(1-methyl-*trans*-4-propyl-L-pyrrolidin-2-yl-carbonylamino)-D-galactose dimethyl dithioacetal was unsuccessful,<sup>29</sup> as was a similar attempt to obtain the methyl *N*-acetyl thiogalactoside.<sup>30</sup>

6-Amino-6-deoxy-1,2,3,4-di-*O*-isopropylidene- $\alpha$ -D-galactopyranose<sup>31</sup> was *N*-acetylated and hydrolysed to the free 6-acetamido-6-deoxy-D-galactose;<sup>30,32</sup> acetylation in pyridine-acetic anhydride gave a mixture of anomeric acetates from which 6-acetamido-1,2,3,4-tetra-*O*-acetyl-6-deoxy- $\beta$ -D-galactose (XXII) was obtained crystalline.

The  $\beta$ -anomer, or the crude mixture of anomers, was converted into the syrupy  $\alpha$ -acetobromo-derivative (XXIII) by reaction with hydrogen bromide in acetic acid, and thence into the crystalline methyl 6-acetamido-2,3,4-tri-*O*-acetyl-6-deoxy-1-thio- $\beta$ -D-galactopyranoside (XXIV) by the Cerny-Pacak procedure in acetone solution. Reaction of this product with bromine in chloroform gave the  $\beta$ -acetobromo-derivative (XXV),



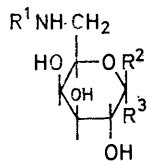
(XXII)  $R^1 = \text{OAc}, R^2 = \text{H}$

(XXIII)  $R^1 = \text{H}, R^2 = \text{Br}$

(XXIV)  $R^1 = \text{SMe}, R^2 = \text{H}$

(XXV)  $R^1 = \text{Br}, R^2 = \text{H}$

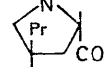
(XXVI)  $R^1 = \text{H}, R^2 = \text{SMe}$



(XXVII)  $R^1 = \text{H}, R^2 = \text{H}, R^3 = \text{SMe}$

(XXVIII)  $R^1 = \text{H}, R^2 = \text{SMe}, R^3 = \text{H}$

(XXIX)  $R^1 = \text{Me}, R^2 = \text{H}, R^3 = \text{SMe}$



(XXX)  $R^1 = \text{Me}, R^2 = \text{SMe}, R^3 = \text{H}$

and reintroduction of the methylthio-group with hexamethylphosphoric triamide as solvent yielded a mixture of the desired  $\alpha$ -thioglycoside (XXVI) and its  $\beta$ -anomer in the ratio ( $\alpha$  :  $\beta$ ) of 1 : 3.5, separated by chromatography.

Hydrazinolysis of the anomeric thioglycosides (XXVI) and (XXIV) yielded the free amino-sugars (XXVII) and (XXVIII), which were condensed with 1-methyl-*trans*-4-propyl-L-proline to give 6-de-(1-hydroxyethyl)-lincomycin (XXIX) and its  $\beta$ -anomer (XXX), isolated as their amorphous hydrochlorides.

**Antibacterial Activities.**—The  $\beta$ -anomeric glycosides (XXI) and (XXX) were found to have less than 0.01% of the activity of lincomycin in *in vitro* tests; the marginal activity of the  $\beta$ -anomers of both lincomycin and 2-deoxylincomycin has been reported.<sup>2</sup> 7-*O*-Methyl-lincomycin (XX) shows the same antibacterial spectrum as lincomycin, and 1.3–1.8 times the *in vitro* activity (standard curve assay against *S. lutea*);

\* Analtech, Inc., Newark, Delaware, U.S.A.

† A saturated hydrocarbon fraction, b.p. 60–71°, Skelly Oil Company, Kansas City, Missouri, U.S.A.

<sup>29</sup> Dr. H. Hoeksema, The Upjohn Company, personal communication.

*in vivo* in the mouse, it shows equivalence to lincomycin in protection against *S. aureus* on subcutaneous injection.

6-De-(1-hydroxyethyl)-lincomycin (XXIX) again shows the same spectrum of activity as lincomycin, but only 1% of the activity *in vitro*. While this activity is low, it may indicate that the function of the side chain is involved with cell-wall permeation, or an increase of binding at an active site.

#### EXPERIMENTAL

M.p.s were determined with a Gallenkamp capillary apparatus. T.l.c. was run on 2 × 8 in Uniplates\* coated with silica gel GF (0.25 mm), in the solvent systems quoted (v/v). Compounds were detected by spraying with the Lemieux reagent,<sup>33</sup> and with 50% aqueous sulphuric acid, followed by heating at 100°. Brinkman silica gel (0.05–0.20 mm) was used for column chromatography. Solvents were removed on a rotatory evaporator at 40° and 7 mmHg. Specific rotations were determined at room temperature for solutions in a 2 dm cell with a Perkin-Stanley polarimeter. I.r. spectra were obtained with a Perkin-Elmer 421 grating spectrometer, for Nujol mulls. Mass spectra were recorded with an Atlas CH-4 spectrometer (direct inlet) at 70 eV.

**Methyl (7S)-N-Acetyl-7-chloro-7-deoxy-3,4-O-isopropylidene-1-thio- $\alpha$ -lincomycin (V).**—Methyl (7S)-N-acetyl-7-chloro-7-deoxy-1-thio- $\alpha$ -lincomycin<sup>5</sup> (5.0 g; finely powdered) was suspended in anhydrous acetone (300 ml), and concentrated sulphuric acid (3.0 ml) was added; the solid dissolved within minutes. After 1.5 h, the solution was made slightly alkaline by passing in dry ammonia, the mixture was filtered from ammonium sulphate, and the filtrate and washings (acetone) were taken to dryness to give a yellow syrup, shown by t.l.c. (methanol-chloroform, 1 : 10) to contain a trace of starting material ( $R_F$  0.20) and a major new zone ( $R_F$  0.26). Chromatography (same solvent) gave a colourless syrup, which afforded crystalline solid (4.36 g; m.p. 124–126°) from acetone-Skellysolve B; † recrystallisation from the same solvent gave plates, m.p. 127–129°, which solidified and then remelted at 146–148° (decomp.),  $[\alpha]_D^{25} + 211^\circ$  ( $c$  0.89 in  $\text{CHCl}_3$ ),  $m/e$  353 ( $M^+$ ) (Found: C, 47.4; H, 6.8; Cl, 10.1; N, 4.0; S, 8.8.  $\text{C}_{14}\text{H}_{22}\text{ClNO}_5\text{S}$  requires C, 47.5; H, 6.8; Cl, 10.0; N, 4.0; S, 9.1%).

**Methyl (6R,7R)-2-O-Acetyl-6,7-acetylpimino-6-deamino-7-deoxy-3,4-O-isopropylidene-1-thio- $\alpha$ -lincomycin (VI).**—A solution of the *N*-acetyl-7-chloroacetamide (V) (1.0 g, 2.84 mmol) in methanol (50 ml) containing sodium methoxide [from sodium (650 mg, 10 mg atom)] was heated under reflux for 18 h. T.l.c. (ethyl methyl ketone-acetone-water, 75 : 25 : 10) showed starting material ( $R_F$  0.72) and a single product ( $R_F$  0.59). Chromatography (same solvent) gave starting material (109 mg, identified by m.p., mixed m.p., and i.r. comparison), and material (600 mg) isolated as a syrup,  $\nu_{\text{max}}$  1705  $\text{cm}^{-1}$ , and distinguished readily by its  $R_F$  (0.59) from the oxazoline acetamide (VIII) ( $R_F$  0.70).<sup>2</sup> Acetylation in pyridine-acetic anhydride gave the product

<sup>30</sup> H. Saeki, T. Iwashige, E. Ohki, K. Furuya, and M. Shirasaka, *Ann. Reports Sankyo Res. Lab.*, 1967, **19**, 137.

<sup>31</sup> K. Freudenberg and A. Doser, *Ber.*, 1925, **58**, 294.

<sup>32</sup> W. A. Szarek and J. K. N. Jones, *Canad. J. Chem.*, 1965, **43**, 2345.

<sup>33</sup> R. U. Lemieux and H. F. Bauer, *Analyt. Chem.*, 1954, **26**, 920.

as *needles* (from ethyl acetate–Skellysolve B), m.p. 145–145.5°,  $[\alpha]_D +189^\circ$  ( $c$  0.81 in  $\text{CHCl}_3$ ),  $m/e$  359 ( $M^+$ ),  $\nu_{\max}$  1745 (ester C=O) and 1705  $\text{cm}^{-1}$  (*N*-acetylpimine) (no amide II band) (Found: C, 53.45; H, 7.0; N, 3.7; S, 8.85.  $\text{C}_{16}\text{H}_{23}\text{NO}_6\text{S}$  requires C, 53.5; H, 7.0; N, 3.9; S, 8.9%).

*Methyl N-Acetyl-2-O-acetyl-3,4-O-isopropylidene-1-thio- $\alpha$ -lincosaminide* (X).—The oxazoline acetonide<sup>2</sup> (VIII) (10.12 g) was acetylated in pyridine–acetic anhydride and the product was isolated as a syrup, which could not be induced to crystallise, but gave only one zone on t.l.c. (ethyl methyl ketone–acetone–water, 75:25:10; starting material  $R_F$  0.72, product 0.79, separated poorly but distinctly). The syrup was heated with water (75 ml) with stirring on a steam-bath, and the hydrolysis was followed by t.l.c. (methanol–chloroform, 1:7). After 2 h, little starting material remained and a major new zone had formed ( $R_F$  0.62) together with a trace of material ( $R_F$  0.23) representing further hydrolysis. Removal of solvent and chromatography (acetone–Skellysolve B, 1:1) gave the desired 7-hydroxy-2-acetate (7.27 g, 60.5%), obtained as *needles* (from acetone–Skellysolve B), m.p. 178–179°,  $[\alpha]_D +194^\circ$  ( $c$  0.73 in  $\text{CHCl}_3$ ),  $m/e$  377 ( $M^+$ ),  $\nu_{\max}$  1745 (ester C=O), 1655 (amide I), and 1520  $\text{cm}^{-1}$  (amide II) (Found: C, 50.7; H, 7.2; N, 3.8; S, 8.5.  $\text{C}_{16}\text{H}_{27}\text{NO}_7\text{S}$  requires C, 50.9; H, 7.2; N, 3.7; S, 8.5%).

*Methyl N-Acetyl-2-O-acetyl-3,4-O-isopropylidene-7-O-methyl-1-thio- $\alpha$ -lincosaminide* (XI).—A mixture of the 7-hydroxy-2-O-acetyl acetonide (X) (1.0 g, 2.65 mmol), methyl iodide (37.6 g, 265 mmol), and silver oxide (3.1 g, 13.25 mmol) was heated under reflux for 16 h. T.l.c. (acetone–Skellysolve B, 1:1) showed the absence of starting material ( $R_F$  0.23) and a major new zone ( $R_F$  0.39), together with several small zones of higher  $R_F$ . Counter-current distribution (500 transfers) in ethanol–water–ethyl acetate–cyclohexane (1:1:1:2) gave the major material ( $K^*$  0.34) as a peak fitting the theoretical curve well, but showing two poorly separated zones on t.l.c. (acetone–Skellysolve B, 1:1). Removal of solvent gave a syrup (650 mg) from which fractional crystallisation from ethyl acetate–Skellysolve B gave the 7-O-methyl 2-acetate (120 mg) as *needles*, m.p. 153.5–154°,  $[\alpha]_D +188^\circ$  ( $c$  1.19 in  $\text{CHCl}_3$ ),  $\nu_{\max}$  1745 (ester C=O), 1650 (amide I), and 1555  $\text{cm}^{-1}$  (amide II),  $m/e$  391 ( $M^+$ ) 332 ( $M^+ - \text{CH}_3 - \text{CHOMe}$ ) (Found: C, 52.2; H, 7.5; N, 3.9; S, 8.0.  $\text{C}_{17}\text{H}_{29}\text{NO}_7\text{S}$  requires C, 52.15; H, 7.5; N, 3.6; S, 8.2%).

The second component, slightly less polar, crystallised from benzene–cyclohexane in prisms, m.p. 124.5–126° (400 mg), identical (m.p., mixed m.p., and i.r. spectra) with methyl *N*-acetyl 3,4-O-isopropylidene-2,7-di-O-methyl-1-thio- $\alpha$ -lincosaminide.<sup>2</sup>

*Methyl N-Acetyl-2,3,4-tri-O-acetyl-7-O-methyl-1-thio- $\alpha$ -lincosaminide* (*Methyl N-Acetyl-2,3,4-tri-O-acetyl-1-thio- $\alpha$ -celestosaminide*) (XIII).—The 7-O-methyl-2-O-acetyl acetonide (100 mg) was stirred with water (20 ml) and aqueous hydrochloric acid (N; 5 ml); after 20 h, t.l.c. (acetone–Skellysolve B, 1:1) showed the absence of starting material ( $R_F$  0.39) and only a zone at the origin was detected. The solution was neutralised by stirring with an excess of silver carbonate (3 g), the solid was removed and washed with water, and the filtrate and washings were lyophilised. Acetylation of the amorphous residue in pyridine–acetic anhydride overnight and isolation in the normal way gave a solid (85 mg) which separated from ethyl acetate–Skellysolve B in *prisms*, m.p. 211.5–213°  $[\alpha]_D +229^\circ$  ( $c$

0.72 in  $\text{CHCl}_3$ ),  $m/e$  435 ( $M^+$ ),  $\nu_{\max}$  1755 (ester C=O), 1660 (amide I), and 1565sh and 1555  $\text{cm}^{-1}$  (amide II) (Found: C, 49.7; H, 6.8; N, 3.4; S, 7.3; OMe, 7.1.  $\text{C}_{18}\text{H}_{29}\text{NO}_8\text{S}$  requires C, 49.6; H, 6.7; N, 3.2; S, 7.4; OMe, 7.1%).

*2-Acetoxyethyl N-Acetyl-2,3,4-tri-O-acetyl-1-thio- $\alpha$ -celestosaminide* (XV).—2-Hydroxyethyl 1-thio- $\alpha$ -celestosaminide<sup>4</sup> (20.0 g) in pyridine (250 ml) and acetic anhydride (120 ml) was left overnight at room temperature, and volatile material was distilled off *in vacuo*. The residual syrup was dissolved in chloroform, washed consecutively with water, dilute hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate, and water, and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of solvent gave a syrup which crystallised from ethyl acetate–Skellysolve B in *prisms* (30.1 g, 88%), m.p. 143–144°  $[\alpha]_D +216^\circ$  ( $c$  0.77 in  $\text{CHCl}_3$ ),  $m/e$  507 ( $M^+$ ),  $\nu_{\max}$  3280 (NH), 1740 (ester C=O), 1650 (amide I), and 1560  $\text{cm}^{-1}$  (amide II) (Found: C, 49.7; H, 6.5; N, 2.9; S, 6.3.  $\text{C}_{21}\text{H}_{33}\text{NO}_{11}\text{S}$  requires C, 49.7; H, 6.5; N, 2.8; S, 6.3%).

*Methyl N-Acetyl-2,3,4-tri-O-acetyl-7-O-methyl-1-thio- $\alpha$ -and - $\beta$ -lincosaminides* (XIII) and (XVII).—A solution of bromine (5.05 g, 31.6 mmol) in chloroform (hydrocarbon-stabilised; 100 ml) was added during 30 min to a stirred solution of the penta-acetate (XV) (10.0 g, 19.8 mmol) in chloroform (200 ml). Initially, the bromine was decolourised immediately, but a deep orange colour persisted later. After an additional 30 min, solvent was removed, giving an orange distillate and yellow residue; more solvent was added and removed until the residue was colourless. This product was dissolved in dry hexamethylphosphoric triamide (50 ml), thiourea (4.5 g, 59.5 mmol) was added, and the colourless solution was stored at room temperature overnight. After cooling to 0°, water (50 ml) was added slowly, with stirring, followed by potassium carbonate (anhydrous; 8.3 g), sodium hydrogen sulphite (10.6 g), and methyl iodide (28.0 g, 198 mmol), and the mixture was stirred vigorously for 3 h.

Volatile material was removed, finally at 110° and <1 mmHg, and the yellow residue was dissolved in chloroform, washed with water, and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of solvent left an amorphous residue (8.3 g), t.l.c. of which showed a major zone (acetone–Skellysolve B, 1:1) of  $R_F$  0.43, and a minor one of  $R_F$  0.48. Chromatography (acetone–Skellysolve B, 1:1) gave the faster-moving component, which crystallised from ethyl acetate–Skellysolve B in *prisms*, m.p. 212–213°, identical with the product (XIII) obtained by the 7-O-methylation procedure (mixed m.p., i.r. spectra). Next was eluted a mixture of both components (660 mg), followed by the slower-moving component (3.25 g); this  $\beta$ -anomer (XVII) crystallised from ethyl acetate–Skellysolve B in *prisms*, m.p. 187–188°,  $[\alpha]_D +24^\circ$  ( $c$  0.75 in  $\text{CHCl}_3$ ),  $m/e$  435 ( $M^+$ ),  $\nu_{\max}$  1760sh, 1745 (ester C=O), 1645 (amide I), and 1570  $\text{cm}^{-1}$  (amide II) (Found: C, 49.7; H, 6.95; N, 3.2; S, 7.6; OMe, 7.4.  $\text{C}_{18}\text{H}_{29}\text{NO}_8\text{S}$  requires C, 49.6; H, 6.7; N, 3.2; S, 7.4; OMe, 7.1%).

When the foregoing reaction sequence was conducted in acetone in place of hexamethylphosphoric triamide, the sole product was the  $\beta$ -anomer (XVII) (86%). When the  $\beta$ -anomer (XVII) replaced the 2-acetoxyethyl thioglycoside (XV) under the hexamethylphosphoric triamide conditions, the same ratio of anomeric methylthio-products resulted.

*Methyl 7-O-Methyl-1-thio- $\alpha$ -lincosaminide* (XVIII).—The tetra-acetate (XIII) (1.46 g) was heated under reflux

\* See ref. 2 for a definition of  $K^*$ .

overnight in hydrazine hydrate (50 ml), volatile materials were distilled off at 110° and 15 mmHg, the residue was triturated with acetonitrile, and the solid was filtered off. The amino-sugar (XVIII) (700 mg, 78.5%), separated from ethanol in flattened *needles* of a hemihydrate, m.p. 126—126.5°,  $[\alpha]_D +263^\circ$  (*c* 0.83 in H<sub>2</sub>O), *m/e* 267 (*M*<sup>+</sup>) (Found: C, 43.6; H, 8.3; N, 5.2; S, 11.7; OMe, 11.7. C<sub>10</sub>H<sub>21</sub>NO<sub>5</sub>·1/2H<sub>2</sub>O requires C, 43.5; H, 8.0; N, 5.1; S, 11.6; OMe, 11.2%).

**7-O-Methyl-lincomycin Hydrochloride (XX).**—To a suspension of 1-methyl-*trans*-4-propyl-L-proline hydrochloride<sup>11</sup> (3.08 g, 14.7 mmol) in anhydrous acetonitrile (75 ml) was added triethylamine (6.56 g, 64.7 mmol), and the solution was cooled to -5° in ice-methanol; a precipitate of triethylammonium chloride appeared. Isobutyl chloroformate (2.02 g, 29.4 mmol) was added at such a rate that the temperature remained between -5 and 0°. After a further 20 min at -5°, a solution of the amino-sugar (XVIII) (2.0 g, 7.35 mmol) in water (25 ml) was added, and the solution was stirred at 0° for 1 h. T.l.c. (ethyl acetate-acetone-water, 9:5:1) showed the presence of a trace of amino-sugar (*R<sub>F</sub>* 0.12) and a major new zone (*R<sub>F</sub>* 0.45). Volatile solvent was removed, and the aqueous residue was adjusted to pH 10 with aqueous sodium hydroxide (N), extracted with chloroform, washed with water, and dried (Na<sub>2</sub>SO<sub>4</sub>). Removal of solvent gave an amorphous solid which was chromatographed in methanol-chloroform (1:15). The desired product was obtained as a syrup (2.20 g) which was dissolved in water (5 ml) by stirring and adding aqueous hydrochloric acid (N) (to pH 3), and the solution was filtered. The filtrate was cooled in ice-methanol, stirred, and diluted with acetone (200 ml) and ether (100 ml), giving the *hydrochloride* of (XX) as elongated platelets (1.71 g), m.p. 155—157°,  $[\alpha]_D +145^\circ$  (*c* 1.06 in H<sub>2</sub>O),  $\nu_{\max}$  1675 (amide I) and 1575 cm<sup>-1</sup> (amide II), *m/e* 420 (*M*<sup>+</sup> of free base) [Found (corrected for 4.83% water): C, 50.1; H, 8.2; Cl, 7.5; N, 6.0; S, 7.2; OMe, 7.0. C<sub>19</sub>H<sub>36</sub>N<sub>2</sub>O<sub>6</sub>S·HCl requires C, 49.9; H, 8.2; Cl, 7.8; N, 6.1; S, 7.0; OMe, 6.8%].

**Methyl 7-O-Methyl-1-thio-β-lincosaminide (XIX).**—Hydrazinolysis of the β-tetra-acetate (XVII) (3.09 g) as before gave the amino-sugar (XIX) as *needles* (970 mg) (from ethanol), m.p. 187—188°,  $[\alpha]_D -7^\circ$  (*c* 0.63 in H<sub>2</sub>O), *m/e* 267 (*M*<sup>+</sup>) (Found: C, 44.7; H, 8.1; N, 5.4; S, 11.3; OMe, 11.4. C<sub>10</sub>H<sub>21</sub>NO<sub>5</sub> requires C, 44.9; H, 7.9; N, 5.2; S, 12.0; OMe, 11.6%).

**7-O-Methyl-β-lincomycin Hydrochloride (XXI).**—The amino-sugar (XIX) (2.0 g) was condensed with 1-methyl-*trans*-4-propyl-L-proline, as before. Chromatography in methanol-chloroform (1:15) gave a syrup (2.38 g), which was converted into the hydrochloride. This could not be obtained crystalline, but was isolated as the amorphous *salt* by lyophilisation of its aqueous solution,  $[\alpha]_D -45^\circ$  (*c* 0.79 in H<sub>2</sub>O),  $\nu_{\max}$  1675 (amide I) and 1565 cm<sup>-1</sup> (amide II), *m/e* 420 (*M*<sup>+</sup> of free base) [Found (corrected for 4.61% water): C, 49.8; H, 8.2, Cl, 7.6; N, 5.7; S, 7.1; OMe, 7.0. C<sub>19</sub>H<sub>36</sub>N<sub>2</sub>O<sub>6</sub>S·HCl requires C, 49.9; H, 8.2; Cl, 7.8; N, 6.1; S, 7.0; OMe, 6.8%].

**6-Acetamido-1,2,3,4-tetra-O-acetyl-6-deoxy-β-D-galactopyranose (XXII).**—6-Acetamido-6-deoxy-1,2,3,4-di-O-isopropylidene-α-D-galactose, obtained by the method of Szarek and Jones,<sup>32</sup> was isolated as rods (from ethyl acetate-Skellysolve B), m.p. 78—79°,  $[\alpha]_D -5^\circ$  (*c* 0.87 in CHCl<sub>3</sub>) (lit.<sup>32</sup> glass,  $[\alpha]_D -8.3^\circ$ ; lit.<sup>30</sup> m.p. 77—79°, no rotation recorded), and was hydrolysed to 6-acetamido-

6-deoxy-D-galactose,<sup>32</sup> isolated as a glass. Acetylation in pyridine-acetic anhydride gave a syrup which crystallised from ethyl acetate-Skellysolve B in irregular *plates*, m.p. 130—131°,  $[\alpha]_D +39^\circ$  (*c* 0.60 in CHCl<sub>3</sub>), *m/e* 389 (*M*<sup>+</sup>),  $\nu_{\max}$  1780, 1755 (ester C=O), 1635 (amide I), and 1580 cm<sup>-1</sup> (amide II) (Found: C, 49.5; H, 6.0; N, 3.6. C<sub>16</sub>H<sub>23</sub>NO<sub>10</sub> requires C, 49.35; H, 5.95; N, 3.6%). T.l.c. (acetone-Skellysolve B, 1:1) showed the crude syrupy product to consist of the β-anomer (*R<sub>F</sub>* 0.22), contaminated with the presumed α-anomer (*R<sub>F</sub>* 0.25).

**Methyl 6-Acetamido-2,3,4-tri-O-acetyl-6-deoxy-1-thio-β-D-galactopyranoside (XXIV).**—The crude syrupy penta-acetate (XXII) (8.55 g, 22.0 mmol) containing the α-anomer was added to a solution of anhydrous hydrogen bromide in glacial acetic acid (40 ml; saturated at room temperature), and stirred until all the solid had dissolved. After 3 h, the viscous solution was diluted with chloroform (200 ml) and poured on ice. The chloroform layer was removed, washed with cold water till the washings were neutral (Congo Red), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to give a syrup (XXIII). To this were added thiourea (5.0 g, 66.0 mmol) and acetone (100 ml); the solution was heated under gentle reflux for 3 h, then cooled in ice. Potassium carbonate (anhydrous; 5.2 g), sodium hydrogen sulphite (6.6 g), water (50 ml), and methyl iodide (10 ml; large excess) were added, and the mixture was stirred vigorously for 2 h.

Removal of the volatile solvent gave an aqueous residue which was extracted with chloroform; the extract was washed with water, and dried (Na<sub>2</sub>SO<sub>4</sub>). T.l.c. (acetone-Skellysolve B, 1:1) showed one product only (*R<sub>F</sub>* 0.34). Removal of the solvent gave a syrup (6.67 g) which crystallised from ethyl acetate-Skellysolve B in *prisms* (5.4 g, 65%), m.p. 116—117°,  $[\alpha]_D +27^\circ$  (*c* 1.02 in CHCl<sub>3</sub>), *m/e* 377 (*M*<sup>+</sup>) and 330 (*M*<sup>+</sup> - SMe),  $\nu_{\max}$  1745, 1725 (ester C=O), 1675 (amide I), and 1535 cm<sup>-1</sup> (amide II) (Found: C, 47.6; H, 6.1; N, 3.9; S, 8.7. C<sub>15</sub>H<sub>23</sub>NO<sub>8</sub>S requires C, 47.7; H, 6.1; N, 3.7; S, 8.5%).

**Methyl 6-Acetamido-2,3,4-tri-O-acetyl-6-deoxy-1-thio-α-D-galactopyranoside (XXVI).**—Bromine (14.65 g, 91.8 mmol) in chloroform (hydrocarbon-stabilised; 200 ml) was added slowly to a stirred solution of the acetylated β-thioglycoside (XXIV) (21.59 g, 57.4 mmol) in chloroform (200 ml). After 1 h, solvent was removed, replaced, and removed again until the residue was colourless. This residue was dissolved in hexamethylphosphoric triamide (200 ml) and the methylthio-group was introduced as described for the 7-O-methyl series. The crude product showed the presence of two materials on t.l.c., the major of *R<sub>F</sub>* 0.17 and not separated from the β-thioglycoside (XXIV), the minor of *R<sub>F</sub>* 0.23 (acetone-Skellysolve B, 1:1). Chromatography gave the α-anomer (3.26 g) followed by a mixture of anomers (2.42 g) and the β-anomer (11.34 g), the reaction giving the mixture of α- and β-products in an overall yield of 79.6%.

The α-anomer (XXVI) crystallised from ethyl acetate-Skellysolve B in elongated *platelets*, m.p. 162—163°,  $[\alpha]_D +207^\circ$  (*c* 1.0 in CHCl<sub>3</sub>),  $\nu_{\max}$  1745 (ester C=O), 1655sh, 1640 (amide I), and 1565 cm<sup>-1</sup> (amide II), *m/e* 377 (*M*<sup>+</sup>) and 330 (*M*<sup>+</sup> - SMe) (Found: C, 48.0; H, 6.5; N, 3.8; S, 8.7. C<sub>15</sub>H<sub>23</sub>NO<sub>8</sub>S requires C, 47.7; H, 6.1; N, 3.7; S, 8.5%).

**6-De-(1-hydroxyethyl)lincomycin Hydrochloride [Methyl 6-Deoxy-6-(1-methyl-*trans*-4-propyl-L-pyrrolidin-2-ylcarbonylamino)-1-thio-α-D-galactopyranoside] (XXIX).**—The

