Modifications of Lincomycin involving the Carbohydrate Portion. Part I. The 2-*O*-Methyl and 2-Deoxy-analogues

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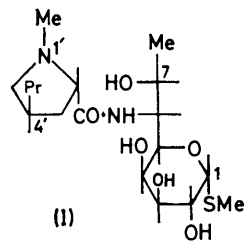
2-*O*-Methyl-lincomycin has been synthesised *via* the *O*-methylation of either methyl *N*-acetyl-3,4-*O*-isopropylidene-1-thiolincosaminide or its 6-deamino-7-deoxy-2'-methyl-[6,7-*d*]-$\Delta^{2'}$-oxazoline derivative, obtained from methyl thiolincosaminide, the carbohydrate portion of the antibiotic lincomycin. The glycal obtained by treatment of either of the anomeric *N*-acetyl-2,3,4,7-tetra-*O*-acetyl-lincosaminyl bromides with zinc, reacts normally with hydrogen bromide to give the anomeric *N*-acetyl-3,4,7-tri-*O*-acetyl-2-deoxylincosaminyl bromides, thereby providing a route to 2-deoxy-α- and β-lincomycins. The effect of the structural modifications in these analogues on antibacterial activity is discussed.

PREVIOUS reports have described the structure [1] and spectrum of antibacterial activity [2] of the antibiotic lincomycin † (I). Modifications of this structure at positions 1' and 4' in the proline ring, and at position 7 in the carbohydrate side chain, have yielded analogues in which the activity essentially is retained or, in some cases, enhanced, and are the subject of a recent review.[3]

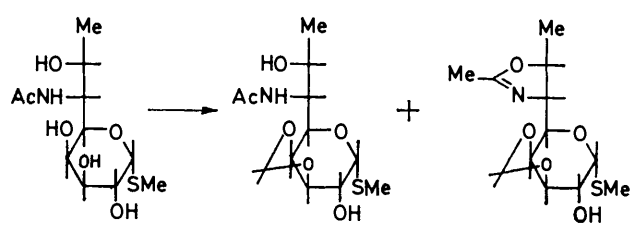


(I)

Replacement of the methylthio-substituent at position 1 by hydrogen [1a] and by hydroxy,[4,5] inversion of its configuration from axial to equatorial,[4] oxidation to the sulphoxide,[5] and conversion to the acyclic dimethyl thioacetal [1c] all result in products showing very low activity. The drastic loss of activity attendant upon these latter changes, together with the observation that replacement of the methylthio-group by ethyl-[6] or n-butyl-thio-[7] gives slight enhancement of activity, prompted an investigation of the environmental requirements of the methylthio-group for activity. For this purpose, conversion of the hydroxy-group at position 2 into methoxy-, and its removal to give the 2-deoxy-analogue, were investigated.

Since *O*-methylation without concomitant quaternisation of the proline nitrogen atom could not be expected in the intact antibiotic, modification was sought at the stage of the amino-sugar. Methyl thiolincosaminide, readily available by hydrazinolysis of lincomycin, was converted into the *N*-acetyl derivative (II),[1c] and treated in acetone with concentrated sulphuric acid at room temperature. Two products were obtained, and separated by chromatography. The more polar component was the 3,4-*O*-isopropylidene derivative (III), reported earlier;[1c] analytical figures for the less polar material corresponded to those for the *N*-acetylacetonide minus a molecule of water. The i.r. spectrum showed hydroxy-absorption and an apparent amide I band at 1650 cm^{-1}, but no amide II band. Since the absence of absorption at 1700 cm^{-1} precluded the formation of an *N*-acetylepimine,[8] an oxazoline structure was indicated, and the compound subsequently was shown to have the structure (IV). The proportion of the oxazoline formed increased with increased time of reaction.

Initially, the stereochemistry at C-7 was in doubt in the structure (IV), since it seemed equally possible that



(II) (III) (IV)

sulphate ester formation at C-7 could occur, followed by displacement by the amide carbonyl group with inversion to give system (b), or that the C-7 hydroxy-group could attack the protonated amide carbonyl group, giving retention of configuration as in system (a) (Scheme). The situation was resolved by the observation that the oxazoline ring opened readily on warming in neutral aqueous solution to give the *N*-acetyl compound (III). Since opening under these conditions must

† Lincocin is the trade-mark of The Upjohn Company for lincomycin hydrochloride.

[1] (*a*) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. S. Schroeder, G. Slomp, and R. R. Herr, *J. Amer. Chem. Soc.*, 1964, **86**, 4223; (*b*) R. R. Herr and G. Slomp, *ibid.*, 1967, **89**, 2444; (*c*) W. Schroeder, B. Bannister, and H. Hoeksema, *ibid.*, p. 2448; (*d*) G. Slomp and F. A. MacKellar, *ibid.*, p. 2454; (*e*) B. J. Magerlein, R. D. Birkenmeyer, R. R. Herr, and F. Kagan, *ibid.*, p. 2459.

[2] D. J. Mason, A. Dietz, and C. DeBoer, *Antimicrobial Agents and Chemotherapy*, 1962, 554; R. R. Herr and M. E. Bergy, *ibid.*, p. 560; L. J. Hanka, D. J. Mason, M. R. Burch, and R. W. Trieck, *ibid.*, p. 565; C. Lewis, H. W. Clapp, and J. E. Grady, *ibid.*, p. 570.

[3] B. J. Magerlein, *Adv. Appl. Microbiol.*, 1971, **14**, 185.

[4] B. Bannister, Abstracts 5th Intersci. Conf. Antimicrobial Agents and Chemotherapy, 1965, p. 17 (details recorded in the Experimental section of this report).

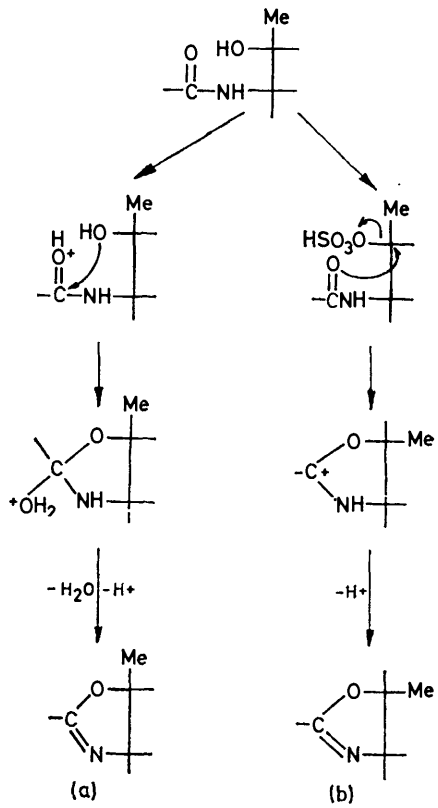
[5] A. D. Argoudelis and D. J. Mason, *J. Antibiotics*, 1969, **22**, 289.

[6] A. D. Argoudelis and D. J. Mason, *Biochemistry*, 1965, **4**, 704.

[7] B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *Antimicrobial Agents and Chemotherapy*, 1966, p. 727.

[8] H. L. Spell, *Analyt. Chem.*, 1967, **39**, 185.

occur by the attack of water at the imino-carbon atom, the stereochemistry at C-7 in the oxazoline must be unchanged, and the oxazoline is methyl 6-deamino-7-deoxy-3,4-*O*-isopropylidene-2′-methyl-1-thiolincosaminido[6,7-*d*]-$\Delta^{2'}$-oxazoline.

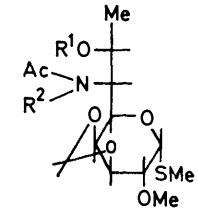


SCHEME

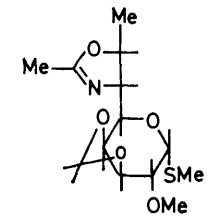
O-Methylation of the acetonide (III) with 1 equiv. of potassium t-butoxide in benzene and methyl iodide, was shown by t.l.c. to yield a mixture which was separated by countercurrent distribution. The most polar substance was a small amount of starting material. The major product, a monomethyl derivative, was identified as the 2-methoxy-compound (V) on the basis of n.m.r. data: a doublet at δ ($CDCl_3$) 2·98 and an associated multiplet at δ 3·95 both collapsed on addition of deuterium oxide, owing to the simplified splitting of the C-7 proton signal by the terminal methyl group. The two other, minor products isolated were shown by analytical and spectral data to be the 2,7-di-*O*-methyl derivative (VI) (two OMe groups, i.r. amide I and II bands) and the *N*-methyl-2,7-di-*O*-methyl derivative (VII) (NMe and two OMe groups, i.r. amide I band, no amide II). Further, the similar *O*-methylation of the oxazoline acetonide (IV) gave a chromatographically pure syrupy product (VIII) in high yield, which was hydrolysed in warm water to the mono-*O*-methyl derivative (V).

Hydrolysis of the 2-*O*-methyl acetonide (V) in dilute hydrochloric acid yielded the 2-*O*-methyltriol amide (IX), which underwent hydrazinolysis to the free 2-*O*-methyl amino-sugar (X). Further demonstration of the presence of the *O*-methyl group at C-2 was obtained from the mass spectrum, which showed *m/e* 267 (M^+) and a base peak at *m/e* 222, due to cleavage between C-6 and C-7 with formation of the stable iminium ion (XI).

Condensation of the amino-sugar (X) with 1-methyl-*trans*-4-propyl-L-proline *via* its mixed anhydride [1c] from isobutyl chloroformate gave 2-*O*-methyl-lincomycin (XII), isolated as its crystalline hydrochloride. Hydrolysis of the 2,7-di-*O*-methylacetonide (VI) and the

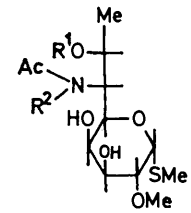


(V) $R^1=R^2=H$
(VI) $R^1=Me, R^2=H$
(VII) $R^1=R^2=Me$

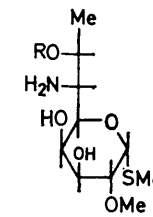


(VIII)

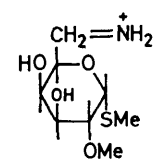
N-methyl-di-*O*-methylacetonide (VII) gave the corresponding *N*-acetyl diols (XIII) and (XIV), respectively, but it was not possible to convert these into the lincomycin analogues; hydrazinolysis of the *N*-acetyl-di-*O*-methyl derivative (XIII) gave the free amino-sugar (XV), which did not react with the mixed 1-methyl-4-propylproline carbonic anhydride; no recognisable product was isolated from the hydrazinolysis of the *N*-acetyl-*N*-methyl-di-*O*-methyl diol (XIV).



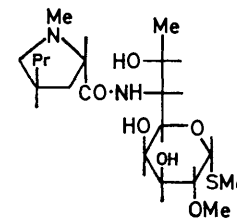
(IX) $R^1=R^2=H$
(XIII) $R^1=Me, R^2=H$
(XIV) $R^1=R^2=Me$



(X) R = H
(XV) R = Me



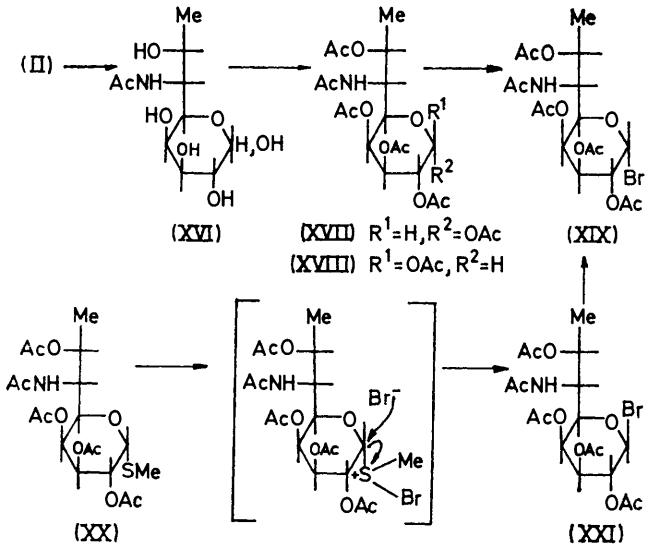
(XI)



(XII)

The simplest access to the 2-deoxy-analogue appeared to be through the glycal derivative of the sugar. Acetylated glycals are available in general by the elimination

of the elements of acetyl hypobromite, in the presence of zinc, from an acetylated glycopyranosyl bromide. Two methods were used to obtain the requisite bromide. The reaction of methyl *N*-acetyl-1-thiolincosaminide (II) in aqueous solution with mercury(II) chloride proceeded slowly, even at 40°, to yield *N*-acetyl-lincosamine (XVI), which was acylated in acetic anhydride–zinc chloride to the hexa-acetyl-α-lincosamine (XVII) or in acetic anhydride–pyridine to a mixture of the α- (XVII) and β- (XVIII) anomers, separated by countercurrent distribution. Both anomers reacted smoothly with a saturated solution of hydrogen bromide in acetic acid to give the crystalline penta-acetyl-α-lincosaminyl bromide (XIX). Alternatively, and more simply, treatment of methyl *N*-acetyl-2,3,4,7-tetra-*O*-acetyl-1-thiolincosaminide [1c] (XX) in chloroform with

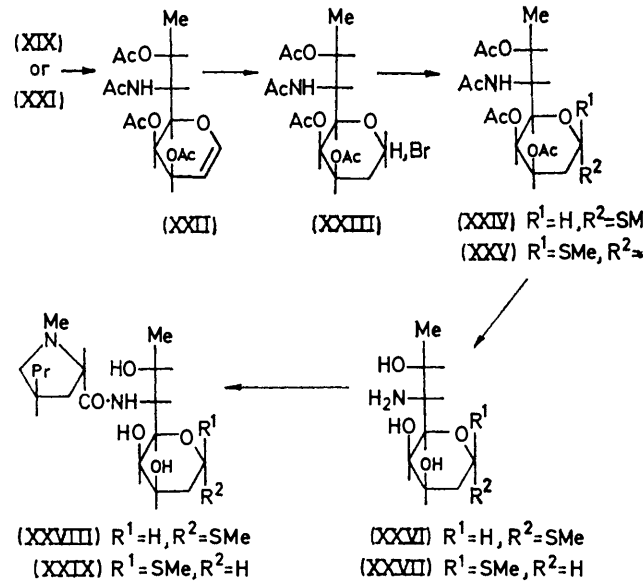


bromine [1c,9] gave, as a colourless syrup, a product considered to be the β-bromide (XXI) on the basis of its rotation and its conversion into the α-anomer (XIX) on treatment with hydrogen bromide in acetic acid.

Both of the anomeric bromides reacted with zinc dust in acetic acid, catalysed by chloroplatinic acid,[10] to give the crystalline tetra-*O*-acetyl-lincosaminal (XXII). The readiness with which such eliminations take place by either bimolecular or unimolecular mechanisms has been remarked upon in the steroid field, where both *trans*- and *cis*-eliminations occur with equal ease.[11] The addition of hydrogen bromide to the glycal, in benzene solution, gave the 2-deoxypyranosyl bromide (XXIII). There was no indication in this reaction of the formation of 3-bromo-2,3-dideoxy-derivatives, found to be the predominant products of the addition of hydrogen bromide to tri-*O*-acetyl-D-glucal [12] and di-*O*-acetyl-D-xylal.[13] The present results are consistent with the formation of the 1-bromo-2-deoxy-derivative as the major product from di-*O*-acetyl-D-arabinal,[13] and lend further support to the suggestion [13,14] that the formation of the 2,3-unsaturated intermediate leading to the 3-halogeno-2,3-dideoxy-derivatives assumes importance only when the departure of the group at C-3 is assisted by the presence of a *trans*- C-4 ester. Treatment of the bromide (XXIII) with thiourea in acetone, hydrolysis of the isothiouronium salt, and methylation of the resulting thiol with methyl iodide [15] gave a mixture of the anomeric methyl *N*-acetyl-tri-*O*-acetyl-2-deoxy-1-thiolincosaminides (XXIV) and (XXV) in the ratio (α : β) of 2 : 1. This is in marked contrast to the formation of the β-anomer exclusively [1c,4] under the same reaction conditions from the 2-acetoxy-α- and β-bromides (XIX) and (XXI), in which participation by the equatorial 2-ester group does not allow the attack of the thiourea from the α-face.

Hydrazinolysis of the anomeric acetylated thioglycosides and condensation of the resulting aminosugars (XXVI) and (XXVII) with 1-methyl-*trans*-4-propylproline gave 2-deoxylincomycin (XXVIII) and its β-anomer (XXIX), isolated as their hydrochlorides.

Antibacterial Activities.—2-Deoxylincomycin shows the same spectrum of antibacterial activity as lincomycin,



but is only 1% as active. Its β-anomer and 2-*O*-methyl-lincomycin show marginal activity only. Lincomycin has been shown [16] to inhibit incorporation of

[9] W. A. Bonner, *J. Amer. Chem. Soc.*, 1948, **20**, 770; F. Weygand and H. Ziemann, *Annalen*, 1962, **657**, 179; M. L. Wolfrom and W. Groebke, *J. Org. Chem.*, 1963, **28**, 2986.

[10] R. E. Deriaz, W. G. Overend, M. Stacey, E. G. Teece, and L. F. Wiggins, *J. Chem. Soc.*, 1949, 1879.

[11] D. R. James, R. W. Rees, and C. W. Shoppee, *J. Chem. Soc.*, 1955, 1370.

[12] T. Maki and S. Tejima, *Chem. and Pharm. Bull.* (*Japan*), 1967, **15**, 1069.

[13] K. Bock, J. Lundt, and C. Pederson, *Acta Chem. Scand.*, 1969, **23**, 2083.

[14] R. J. Ferrier, R. D. Guthrie, and T. D. Inch, in 'Carbohydrate Chemistry,' Chem. Soc. Specialist Periodical Report, 1970, vol. 3, pp. 59—60.

[15] M. Cerny, J. Vrkoc, and J. Stanek, *Coll. Czech. Chem. Comm.*, 1959, **24**, 64; M. Cerny and J. Pacak, *ibid.*, p. 2566.

[16] J. E. Grady and F. Reusser, *Progr. Antibacterial Anticancer Chemother.*, 1969, **2**, 468.

amino-acids into proteins at the ribosomal level. With 2-deoxylincomycin, no significant inhibition of incorporation of amino-acids by ribosomes in a cell-free system occurred, showing the low activity of this analogue to be due to its lack of effective interaction at the ribosomal level, and not to a failure to permeate the cell membrane.

These results demonstrate the great influence of the environment of the methylthio-group on the antibacterial activity of the antibiotic, and may imply the requirement of a *cis*-relationship between the methylthio- and hydroxy-groups at positions 1 and 2 for effective 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 [17] or with 50% aqueous sulphuric acid followed by heating at 100°. Brinkmann silica gel (0·05—0·20 mm) for chromatography 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. N.m.r. spectra were recorded for solutions in deuteriochloroform at 60 MHz with a Varian A60A spectrometer (tetramethylsilane as internal standard). Mass spectra were recorded with an Atlas CH-4 spectrometer (direct inlet) at 70 eV.

Reaction of Methyl N-*Acetylthiolincosaminide with Acetone–Sulphuric Acid: Methyl* N-*Acetyl*-3,4-O-*isopropylidene*-1-*thiolincosaminide* (III) *and Methyl* 6-*Deamino*-7-*deoxy*-3,4-O-*isopropylidene*-2′-*methyl*-1-*thiolincosaminido*[6,7-d]-$\Delta^{2'}$-*oxazoline* (IV).—Finely powdered methyl *N*-acetylthiolincosaminide [1c] (16·0 g) was suspended in dry acetone (1·6 l) with vigorous stirring, and concentrated sulphuric acid (16 ml) was added. The solid dissolved during 1 h; after 3 h at room temperature the solution was stored overnight in the refrigerator, and made slightly alkaline by leading in dry ammonia. Filtration and washing of the ammonium sulphate gave a colourless solution yielding a semicrystalline syrup on removal of solvent. Chromatography on silica (ethyl methyl ketone–acetone–water, 75 : 25 : 10) gave the *oxazoline acetonide* (R_F 0·74; 2·4 g), which separated from acetone as needles, m.p. 191—192·5°, $[\alpha]_D$ +126° (*c* 0·85 in EtOH) (Found: C, 52·8; H, 7·3; N, 4·4; O, 25·1; S, 10·1. $C_{14}H_{23}NO_5S$ requires C, 53·0; H, 7·3; N, 4·4; O, 25·2; S, 10·1%).

Further elution gave the *N*-acetylacetonide (R_F 0·58; 4·6 g) with the physical constants reported earlier.[1c] Intermediate fractions were a mixture of the two products (6·1 g) which could be hydrolysed to the *N*-acetylacetonide as described later for the oxazoline acetonide. Continuation of the reaction for a longer period gave an increased proportion of the oxazoline acetonide.

Hydrolysis of the Oxazoline Acetonide (IV) *to the* N-*Acetyl-acetonide* (III).—A solution of the oxazoline (1·0 g) in water (20 ml) was heated under reflux: after 1·5 h, t.l.c. (ethyl methyl ketone–acetone–water, 75 : 25 : 10) showed the disappearance of starting material and the generation of a product not distinguished from the *N*-acetylacetonide. On cooling, the product separated as needles, identical with the *N*-acetylacetonide (m.p., mixed m.p., and i.r. spectra).

Methylation of the N-*Acetylacetonide*.—Potassium (1·16 g, 29·8 mg atom) was dissolved in dry t-butyl alcohol; the solvent was distilled off and replaced by benzene, and the *N*-acetylacetonide (10·0 g, 29·8 mmol) was added with stirring. After 1 h, methyl iodide (84·8 g, 596 mmol) was added. The mixture was neutral after 2 h; filtration and removal of solvent gave a colourless syrup showing starting material and three products on t.l.c. (R_F 0·58, 0·60, and 0·68 in ethyl methyl ketone–acetone–water, 75 : 25 : 10). Countercurrent distribution in the system ethyl acetate–ethanol–water (4 : 1 : 2) yielded, after 500 transfers, starting material (K† 0·39) and products of K 1·30, 2·52, and 5·67. The major material, K 1·30 (4·16 g), the 2-O-*methyl derivative* (V), separated from ethyl acetate–Skellysolve B ‡ as needles, m.p. 176—177°, $[\alpha]_D$ +176° (*c* 0·62 in $CHCl_3$) (Found: C, 51·8; H, 8·1; N, 4·1; S, 8·9; OMe, 8·5. $C_{15}H_{27}NO_6S$ requires C, 51·6; H, 7·8; N, 4·0; S, 9·2; OMe, 8·9%).

The material of K 2·52 (2·16 g), the 2,7-di-*O*-methyl derivative (VI), was isolated as *prisms* from ethyl acetate–Skellysolve B, m.p. 124·5—126°, $[\alpha]_D$ +184° (*c* 0·84 in $CHCl_3$) (Found: C, 53·0; H, 7·95; N, 4·05; S, 8·7; OMe, 15·9. $C_{16}H_{29}NO_6S$ requires C, 52·9; H, 8·0; N, 3·85; S, 8·8; OMe, 17·1%), showing amide I and II bands at $\nu_{max.}$ 1650 and 1560 cm^{-1}.

The material of K 5·67 (790 mg), the *N*-methyl-di-*O*-methyl derivative (VII), crystallized from ether in irregular *platelets*, m.p. 104·5—106°, $[\alpha]_D$ +174° (*c* 0·83 in $CHCl_3$) (Found: C, 54·1; H, 8·2; N, 4·1; S, 8·3; NMe, 8·1; OMe, 16·2. $C_{17}H_{31}NO_6S$ requires C, 54·1; H, 8·3; N, 3·7; S, 8·5; NMe, 7·7; OMe, 16·4), $\nu_{max.}$ (amide I) 1635 cm^{-1}, no amide II band.

Methylation of the Oxazoline Acetonide (IV).—Under the same conditions as before, with 1·5 equiv. of potassium t-butoxide, the oxazoline acetonide (5·0 g) gave a light tan syrup (4·96 g) which could not be induced to crystallize, and which showed absence of starting material on t.l.c. (ethyl methyl ketone–acetone–water, 75 : 25 : 10) and a new less polar zone (R_F 0·73). This syrup (VIII) was heated with water (75 ml) under reflux for 1 h, after which all of the syrup had dissolved, and t.l.c. (same solvent system) showed the disappearance of the R_F 0·73 zone and formation of a zone of R_F 0·60, not separated from the *N*-acetyl-2-*O*-methyl derivative (V). Removal of the water, and crystallization from ethyl acetate–Skellysolve B gave needles (3·90 g), identical (m.p., mixed m.p., and i.r. spectra) with compound (V).

Methyl N-*Acetyl*-2-O-*methyl*-1-*thiolincosaminide* (IX).—The 2-*O*-methyl acetonide (IV) (7·33 g) was dissolved with stirring in dilute hydrochloric acid (0·25 N; 75 ml); after 2 h, t.l.c. (ethyl methyl ketone–acetone–water, 75 : 25 : 10), showed the absence of starting material and the formation of a single more polar zone. Neutralization with Amberlite IRA 400 (OH^- form) and removal of the water left a

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

† The distribution coefficient K is defined by the expression $K = r/(n - r)$ in which r is the tube no. of the centre of the peak and n is the total no. of transfers.

‡ A saturated hydrocarbon fraction, b.p. 60—71°, Skelly Oil Co., Kansa City, Missouri, U.S.A.

[17] R. U. Lemieux and H. F. Bauer, *Analyt. Chem.*, 1954, **26**, 920.

crystalline residue (6·8 g) which separated as felted *needles* from methanol–ether, m.p. 237—238°, $[\alpha]_D$ +270° (*c* 0·69 in 50% H_2O–EtOH) (Found: C, 46·7; H, 7·4; N, 4·4; S, 10·3; OMe, 10·3. $C_{12}H_{23}NO_6S$ requires C, 46·6; H, 7·5; N, 4·5; S, 10·4; OMe, 10·0%).

Methyl N-*Acetyl*-2,7-*di*-O-*methyl*-1-*thiolincosaminide* (XIII).—Hydrolysis of the 2,7-di-*O*-methyl acetonide (6·17 g) was conducted as for the 2-*O*-methyl compound, giving the product as *prisms* (3·28 g) (from ethyl acetate), m.p. 159—160°, $[\alpha]_D$ +272° (*c* 0·71 in 50% H_2O–EtOH) (Found: C, 48·1; H, 7·9; N, 4·3; S, 9·8; OMe, 18·3. $C_{13}H_{25}NO_6S$ requires C, 48·3; H, 7·8; N, 4·3; S, 9·9; OMe, 19·2%).

Methyl N-*Acetyl*-N-*methyl*-2,7-*di*-O-*methyl*-1-*thiolincosaminide* (XIV).—By the same procedure, the trimethyl acetonide (5·93 g) was converted into the diol, obtained as a *syrup* (5·24 g), $[\alpha]_D$ +241° (*c* 0·84 in 50% H_2O–EtOH) (Found: C, 49·7; H, 8·1; N, 3·9; S, 9·35. $C_{14}H_{27}NO_6S$ requires C, 49·8; H, 8·1; N, 4·15; S, 9·5%).

Methyl 2-O-*Methyl*-1-*thiolincosaminide* (X).—The *N*-acetyl-2-*O*-methyl derivative (5·0 g) was heated under reflux with hydrazine hydrate (100 g) overnight; the solvent was distilled off under reduced pressure and the residual solid crystallized from ethanol. The free amine separated as *needles* (3·6 g), m.p. 197—198°, $[\alpha]_D$ +268° (*c* 0·93 in H_2O) (Found: C, 44·9; H, 8·2; N, 5·4; S, 11·9. $C_{10}H_{21}NO_5S$ requires C, 44·9; H, 7·9; N, 5·2; S, 12·0%).

Methyl 2,7-*Di*-O-*Methyl*-1-*thiolincosaminide* (XV).—The *N*-acetyl-2,7-di-*O*-methyl derivative (2·13 g) was hydrazinolysed as before and gave the free amine as *needles* (1·33 g) (from ethanol), m.p. 119—120°, $[\alpha]_D$ +266° (*c* 0·90 in H_2O) (Found: C, 47·2; H, 8·5; N, 5·2; S, 11·5. $C_{11}H_{23}NO_5S$ requires C, 46·9; H, 8·2; N, 5·0; S, 11·4%).

2-O-*Methyl*-*lincomycin Hydrochloride* (XII).—To a slurry of 1-methyl-*trans*-4-propylproline [1c] (2·0 g, 9·6 mmol) in anhydrous acetonitrile (50 ml) was added triethylamine (2·15 g, 21·3 mmol); the solution was cooled to −5° in an ice–methanol bath, and isobutyl chloroformate (1·32 g, 9·6 mmol) was added slowly. After 20 min, a solution of methyl 2-*O*-methyl-1-thiolincosaminide (1·3 g, 4·8 mmol) in water (20 ml) was added, and the reaction was allowed to proceed for 1 h at 0°. Removal of the solvent left a tan aqueous residue which was adjusted to pH 10 with aqueous sodium hydroxide (N), and extracted with chloroform (3 × 100 ml); the extracts were washed with water, and dried (Na_2SO_4). Removal of solvent left a syrup (1·53 g), which was chromatographed on silica in ethyl acetate–acetone–water (8 : 5 : 1).

The product (R_F 0·4; 1·1 g) was dissolved in water (3 ml), acidified to pH 4 with hydrochloric acid, stirred, and diluted with acetone (50 ml), giving a crystalline solid (960 mg; m.p. 252—255°). Recrystallization from water–acetone at 0° gave the *hydrochloride*, needles, m.p. 255—260° (decomp.), $[\alpha]_D$ +140° (*c* 0·88 in H_2O) (Found: C, 49·7; H, 8·1; Cl, 8·1; N, 6·0; S, 7·2; OMe, 7·4. $C_{19}H_{36}N_2O_6S$,HCl requires C, 49·9; H, 8·2; Cl, 7·8; N, 6·1; S, 7·0; OMe, 6·8%).

N-*Acetyl*-1,2,3,4,7-*penta*-O-*acetyl*-α- *and* β-*lincosamines* (XVII) *and* (XVIII).—To a stirred solution of methyl *N*-acetylthiolincosaminide (6·4 g) in water (400 ml) was added a solution of mercury(II) chloride (8·8 g) in water (400 ml): a precipitate of chloro(methylthio)mercury began to appear after several minutes. After 24 h, this precipitate was collected and dried (4·76 g ≡ 77·5% reaction); t.l.c. (butanol–ethanol–water, 5 : 1 : 4) showed starting material (R_F 0·35) and product (R_F 0·19). On warming the filtrate to 40°, further precipitation commenced, and the solution was held at this temperature for 4 days, giving additional chloro(methylthio)mercury (940 mg, total ≡ 92·7% reaction). Excess of mercury(II) chloride was precipitated as its pyridine complex, chloride ion removed with silver carbonate, and the solution was saturated with hydrogen sulphide, filtered, and lyophilised. The residue (XVI) (5·19 g) was acetylated in pyridine (50 ml) and acetic anhydride (25 ml), giving a syrup which was subjected to countercurrent distribution in the system acetone–water–ethyl methyl ketone–cyclohexane (5 : 3 : 4 : 4). After 900 transfers, methyl penta-acetylthiolincosaminide [1c] was isolated (*K* 1·14; 730 mg; m.p. 218—220°); the anomeric hexa-acetates were separated incompletely, but pure materials could be isolated from the extremities of the peaks. The α-anomer (*K* 0·66) (XVII) was obtained from ethyl acetate–Skellysolve B as *platelets*, m.p. 240—240·5°, $[\alpha]_D$ +132° (*c* 0·98 in $CHCl_3$) (Found: C, 50·6; H, 6·1; N, 3·0. $C_{20}H_{29}NO_{12}$ requires C, 50·5; H, 6·15; N, 2·95%). The β-anomer (*K* 0·75) (XVIII) separated from ethyl acetate–Skellysolve B as *needles*, m.p. 230—231°, $[\alpha]_D$ +33° (*c* 0·83 in $CHCl_3$) (Found: C, 50·4; H, 6·4; N, 3·0%). Acetylation of the crude *N*-acetyl-lincosamine (XVI) in acetic anhydride containing anhydrous zinc chloride at 100° for 1 h gave the α-anomer (XVII) only, m.p. 240—241°.

N-*Acetyl*-2,3,4,7-*tetra*-O-*acetyl*-α-*lincosaminyl Bromide* (XIX).—A solution of the α-hexa-acetate (XVII) (2·0 g) in acetic acid saturated at 0° with hydrogen bromide (5 ml) was diluted with chloroform after 2 h and poured on ice, and the chloroform layer was separated, washed with ice–water, and dried (Na_2SO_4). Removal of the solvent and crystallisation from chloroform–Skellysolve B gave *rods* (1·8 g), m.p. 188—189°, $[\alpha]_D$ +231° (*c* 0·81 in $CHCl_3$) (Found: C, 43·7; H, 5·4; Br, 16·4; N, 2·9. $C_{18}H_{26}BrNO_{10}$ requires C, 43·6; H, 5·3; Br, 16·1; N, 2·8%). The β-hexa-acetate (XVIII) behaved identically under these conditions.

N-*Acetyl*-2,3,4,7-*tetra*-O-*acetyl*-β-*lincosaminyl Bromide* (XXI).—The reaction between methyl *N*-acetyl-2,3,4,7-tetra-*O*-acetylthiolincosaminide (XX) and bromine [1c] in chloroform yielded a colourless syrup, which could not be induced to crystallise, $[\alpha]_D$ −6° (*c* 0·71 in $CHCl_3$), converted by treatment with hydrogen bromide in acetic acid into a product identical (m.p. and mixed m.p.) with foregoing α-anomer; therefore the syrup is considered to be the β-anomer (XXI).

N-*Acetyl*-3,4,6-*tri*-O-*acetyl*-*lincosaminal* (XXII).—The β-acetobromo-derivative (XXI) [from the methyl penta-acetylthiolincosaminide (XX) (10·0 g) [1c]] was dissolved in aqueous acetic acid (50%; 120 ml) and added to zinc dust (7 g) with vigorous stirring. Aqueous chloroplatinic acid (3%; 0·5 ml) was added; additional zinc (14 g) and a few drops of chloroplatinic acid were added at intervals during 2 h. After filtration and removal of the solvent from the filtrate, the residue was partitioned between chloroform and water. The organic extract was washed with water, saturated aqueous sodium hydrogen carbonate, and water, and dried (Na_2SO_4). Following solvent removal, the residual solid was chromatographed on silica (acetone–Skellysolve B, 1 : 1). The *glycal* (4·42 g), R_F 0·4 (same solvent) was obtained as needles from ethyl acetate, m.p. 248—250°, $[\alpha]_D$ +94° (*c* 1·09 in $CHCl_3$) (Found: C, 53·8; H, 6·7; N, 4·2. $C_{16}H_{23}NO_8$ requires C, 53·8; H, 6·5; N, 3·9%), δ [$(CD_3)_2N{\cdot}CDO$] 9·47 (1H, d, amide NH), 6·49 (1H, d × 2, H-2), 5·5 (2H, m, H-3 and H-4), 5·19 (1H,

q × 2, H-7), 4·60—4·34 (2H, m, H-1 and H-6), 4·20 (1H, d, H-5), 2·0, 1·92, and 1·83 (12H, s × 3, four acetates), and 1·23 p.p.m. (3H, d, terminal CH_3).

The α-acetobromo-derivative (XIX) reacted under the same conditions to give the glycal in equivalent yield.

Methyl N-*Acetyl*-3,4,7-*tri*-O-*acetyl*-2-*deoxy*-1-*thio*-α- *and* β-*lincosaminides* (XXIV) *and* (XXV).—The tetra-acetyl glycal (2·0 g, 5·6 mmol) in benzene (50 ml) was saturated with hydrogen bromide at 0°; the solvent was removed after 30 min, the amorphous residue was redissolved in benzene, and the solvent was removed again. T.l.c. showed the disappearance of glycal (R_F 0·4) and the formation of a new zone (R_F 0·24) (acetone–Skellysolve B, 1 : 1). The residue was dissolved in acetone (4 ml), thiourea (0·94 g, 8·4 mmol) was added, and the mixture was heated under reflux for 1 h. To the cooled solution were added water (25 ml), potassium carbonate (1·83 g), sodium hydrogen sulphite (2·32 g), and methyl iodide (7·95 g, 56 mmol), and the mixture was stirred vigorously at room temperature for 3 h. T.l.c. (acetone–Skellysolve B, 1 : 1) showed two major zones, R_F 0·40 and 0·47.

Volatile solvent was removed, and the aqueous residue was extracted with chloroform. The extract was washed with water and dried (Na_2SO_4). Countercurrent distribution of the residue (1·24 g) in the system water–acetone–ethyl methyl ketone–cyclohexane (3 : 5 : 4 : 4) gave, after 500 transfers, two well-defined peaks of K 0·81 and 1·43. The crystalline solid (280 mg) obtained from the lower peak gave the β-*anomer* (XXV) as platelets (from ethyl acetate–Skellysolve B), m.p. 207—209°, $[\alpha]_D$ +13° (*c* 0·71 in $CHCl_3$). Absorbance centred at δ 3·57 p.p.m. (1H, t, H-5) supports the β-configuration at C-1 (see under α-anomer) (Found: C, 50·4; H, 6·75; N, 3·3; S, 7·85. $C_{17}H_{27}NO_8S$ requires C, 50·35; H, 6·7; N, 3·5; S, 7·9%).

The solid (540 mg) from the higher peak gave the α-anomer (XXIV) as *needles* from ethyl acetate, m.p. 227—228°, $[\alpha]_D$ +260° (*c* 0·81 in $CHCl_3$); absorbance centred at δ 4·21 p.p.m. (1H, t, H-5) supports the α-configuration, since the 1,3-diaxial interaction between the 5-H and the electronegative SMe would be expected to make the 5-H triplet appear downfield relative to that in the equatorial β-thioglycoside (Found: C, 50·5; H, 6·6; N, 3·7; S, 7·8%).

Methyl 2-*Deoxy*-1-*thio*-α-*lincosaminide* (XXVI).—Hydrazinolysis of the α-anomeric tetra-acetate (XXIV) (6·47 g) gave the amino-sugar as *needles* (3·21 g), m.p. 206—208° (from ethanol), $[\alpha]_D$ +257° (*c* 0·80 in H_2O); *m/e* 237 (M^+) and 192 ($M^+ - CH_3\dot{C}HOH$) (Found: C, 45·4; H, 8·15; N, 6·1; S, 13·25. $C_9H_{19}NO_4S$ requires C, 45·55; H, 8·1; N, 5·9; S, 13·5%).

2-*Deoxylincomycin Hydrochloride* (XXVIII).—Condensation of the amino-sugar (XXVI) (2·0 g) with 1-methyl 4-propylproline by the method used before and chromatography on silica in methanol–chloroform (1 : 7) gave the free base (2·25 g; R_F 0·40), converted in acetone–dilute hydrochloric acid into the hydrochloride, obtained as *needles*, m.p. 165—167° (decomp.), $[\alpha]_D$ +125° (*c* 0·84 in H_2O) [Found (corrected for 2·21% water by Karl Fischer analysis): C, 50·4; H, 8·15; Cl, 8·4; N, 6·5; S, 7·4. $C_{18}H_{34}N_2O_5S$,HCl requires C, 50·6; H, 8·3; Cl, 8·3; N, 6·6; S, 7·5%].

Methyl 2-*Deoxy*-1-*thio*-β-*lincosaminide* (XXVII).—In a process similar to the foregoing hydrazinolysis, the β-tetra-acetate (XXV) (3·14 g) gave the free amino-sugar (XXVII) as *needles* (1·05 g), m.p. 181—183° (from ethanol), $[\alpha]_D$ −38° (*c* 1·1 in H_2O) (Found: C, 44·9; H, 8·3; N, 5·8; S, 13·35%).

2-*Deoxy*-β-*lincomycin Hydrochloride* (XXIX).—Condensation of the amino-sugar (XXVII) (1·87 g) with 1-methyl-4-propylproline under the usual conditions and chromatography on silica in methanol–chloroform (1 : 15) gave the free base (1·15 g) [R_F 0·1 (same system)], converted into the hydrochloride as an amorphous *solid*, $[\alpha]_D$ −64° (*c* 1·02 in H_2O) [Found (corrected for 2·96% water): C, 50·3; H, 8·4; Cl, 8·5; N, 6·7; S, 7·3%].

Methyl 1-*Thio*-β-*lincosaminide*.—Methyl *N*-acetyl-2,3,4,7-tetra-*O*-acetyl-1-thio-β-lincosaminide[1c] (4·0 g) was hydrazinolysed as before; the amino-sugar was obtained from n-butanol as *needles*, m.p. 219·5—221°, $[\alpha]_D$ −7° (*c* 0·75 in H_2O) (Found: C, 42·8; H, 7·3; N, 5·6; S, 13·0. $C_9H_{19}NO_5S$ requires C, 42·7; H, 7·6; N, 5·5; S, 12·7%).

β-*Lincomycin Hydrochloride*.—Condensation of the foregoing β-amino-sugar (4·6 g) with 1-methyl-4-propylproline by the normal procedure and chromatography on silica in methanol–chloroform (1 : 7) gave the free base (4·1 g), converted into the amorphous *hydrochloride*, $[\alpha]_D$ −44° (*c* 1·04 in H_2O) [Found (corrected for 2·31% water): C, 49·0; H, 8·2; Cl, 8·3; N, 6·2; S, 6·9. $C_{18}H_{34}N_2O_6S$,HCl requires C, 48·8; H, 8·0; Cl, 8·0; N, 6·3; S, 7·2%].

1-*Demethylthio*-1-*hydroxylincomycin* (*Lincomycose*).—Bromine (1·42 g, 9 mmol) was added dropwise to a stirred solution of lincomycin hydrochloride (2·0 g, 4·5 mmol) in water (50 ml); the colour was discharged immediately. After 15 min, Amberlite IRA-400 (OH^- form) was added till the pH reached 8·5, and the resin was filtered off and washed with water. T.l.c. (methanol–chloroform–ether, 25 : 50 : 15) showed the absence of lincomycin (R_F 0·44) and the formation of a new zone (R_F 0·17), and the solution gave an immediate precipitate with Benedict's reagent.

The filtrate was lyophilised, and the amorphous residue (1·1 g) was subjected to countercurrent distribution in the system n-butanol–water (1 : 1). After 800 transfers, pure lincomycose, K 0·07, was isolated as an amorphous solid, $[\alpha]_D$ +6° (*c* 0·99 in H_2O), *m/e* 376 (M^+, free base), (no methylthio peak at δ 2·58) δ 5·02br p.p.m. (mixture of anomers) (Found: C, 47·4; H, 8·7; Cl, 8·5; N, 6·5. $C_{17}H_{32}N_2O_7$,HCl,H_2O requires C, 47·4; H, 8·0; Cl, 8·2; N, 6·5%).

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