Modifications of Lincomycin Involving the Carbohydrate Portion. Part IV.[1] (7*S*)-7-Alkoxy-7-deoxy-analogues

By **Brian Bannister**, The Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001, U.S.A.

Modifications of the antibiotic lincomycin have been synthesised in which the 7(*R*)-hydroxy-group of the parent has been replaced by a series of 7(*S*)-alkoxy-substituents. These substituents were introduced by the ready acid-catalysed alcoholysis of the 6,7-acetylepimine (XI) or its 2,3,4-tri-*O*-acetyl ester (XXI), derived from the carbohydrate portion of the antibiotic. Hydrazinolysis yielded the free amino-sugars, and condensation with 1-methyl-*trans*-4-propyl-L-proline gave the lincomycin analogues. The (7*S*)-7-deoxy-7-methoxy-analogue shows significantly enhanced antibacterial activity, but this activity diminishes as larger alkoxy-substituents are introduced.

THE contrast between modifications in the antibiotic lincomycin † [methyl 6,8-dideoxy-6-(1-methyl-*trans*-4-propyl-L-pyrrolidin-2-ylcarbonylamino)-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside] (I) involving positions 1, 2, 3, and 4 in the carbohydrate ring,[2,3] which result in drastic reduction of antibacterial activity, and those at C-7, which may result in increased potency and spectrum of activity, has been noted.[1] Both the nature of the substituent and its stereochemistry are reflected in the activities of such analogues. Thus, the 7(*S*)-epimer (II) is one half as active as lincomycin, the (7*R*)-7-chloro-7-deoxy-analogue (III) is twice as active as lincomycin, and the (7*S*)-7-chloro-7-deoxy-analogue ‡ (IV) is four times as active as lincomycin, and also shows activity against some Gram-negative bacteria.[4] Since the 7-*O*-methyl analogue of lincomycin in which the methoxy-substituent retains the *R*-configuration of the parent shows enhanced antibacterial activity[1] the synthesis of its 7(*S*)-epimer and of related alkoxy-analogues was of interest.

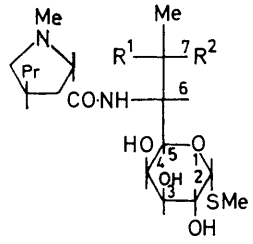
Guthrie and Murphy[5] have suggested that epimino-sugars should be useful intermediates for the synthesis of a variety of α-substituted amino-sugars, but they have also shown that, as distinct from epoxy-sugars, the epimino-sugars do not undergo ring-opening with alkoxide anion. The 6,7-epimine (V)[6] is available readily from methyl thiolincosaminide,[7] and has been shown to

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

‡ Cleocin is the trademark of The Upjohn Company for clindamycin hydrochloride, (7*S*)-7-chloro-7-deoxylincomycin hydrochloride.

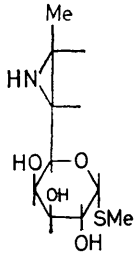
[1] Part III, B. Bannister, *J.C.S. Perkin I*, 1973, 1676.
[2] B. Bannister, *J.C.S. Perkin I*, 1972, 3025.
[3] B. Bannister, *J.C.S. Perkin I*, 1972, 3031.
[4] B. J. Magerlein, *Adv. Appl. Microbiol.*, 1971, **14**, 185.
[5] R. D. Guthrie and D. Murphy, *J. Chem. Soc.*, 1963, 5288.
[6] B. J. Magerlein and F. Kagan, *J. Medicin. Chem.*, 1970, **13**, 616.
[7] W. Schroeder, B. Bannister, and H. Hoeksema, *J. Amer. Chem. Soc.*, 1967, **89**, 2448.

react with hydrogen sulphide in isopropyl alcohol at 100° to give the L-*threo*-amino-thiol (VI).[6] The epimine is completely unreactive towards methanol in a sealed tube at 100°. Since both hydrogen sulphide and alkanethiols are known to effect ring-opening of simple epimines,[8] the implication is that the first step in such reactions is protonation of the epimine nitrogen atom, resulting in weakening of the C–N bond, and is followed by

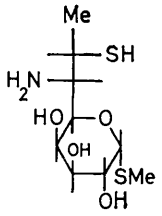


(I) R^1 = OH, R^2 = H
(II) R^1 = H, R^2 = OH
(III) R^1 = Cl, R^2 = H
(IV) R^1 = H, R^2 = Cl

nucleophilic attack by the conjugate base. The lack of reactivity of methanol presumably is due to its acidity being much lower than that of hydrogen sulphide or thiols,[9] together with the nucleophilicity of methoxide ion being lower than that of the hydrogen sulphide or thiolate ions.[10] Indeed, that the ring-opening of an epimine is catalysed by acids was indicated by the observation [11] that methanolysis of the picrate salt of a simple epimine occurred during attempted crystallisation from methanol.



(V)



(VI)

An extremely slow reaction occurred at room temperature between the epimine (V) and methanol in the presence of 1 equiv. of sulphuric acid (restrictions imposed by the relative lability toward strong acid of the thioglycosidic group). The formation of several products after several days was demonstrated by t.l.c., and much starting material was still present. One of the more polar products later was found not to be differentiated from the desired methoxy-amine by t.l.c., but the minor amount of this material precluded its isolation.

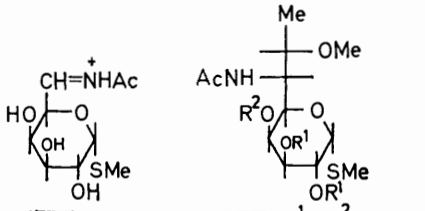
An *N*-acylated epimine similarly would be expected to be more susceptible to ring-opening by a nucleophile than the free epimine and, in epimino-sugars, ring-opening by azide ion has been achieved in 2,3-acylepimino-D-*manno*-,[12] D-*allo*-[12,13] and D-*lyxo*-[14] derivatives. However, Guthrie and Murphy [12] showed that the methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-acetylepimino-α-D-manno- and allo-pyranosides underwent de-*N*-acetylation to give the free epimines prior to ring-opening. Furthermore, the reaction of an acetylepimine with sodium methoxide in methanol results in ready hydrolysis to the free epimine, which is stable to nucleophilic attack by methoxide ion.[12,13,15,16] This enhanced reactivity of the carbonyl group in acylepimines, relative to other tertiary amides, has been attributed [17] to lessened interactions of the ring nitrogen atom with the carbonyl group, reflected in the significantly higher carbonyl stretching frequencies of acylepimines than of acyclic tertiary amides. On the other hand, Hough and Richardson and their co-workers [15,18] have shown that carbohydrate 2,3-acylepimines are more susceptible to ring-opening with hydrogen halides than are the free epimines, and Saeki and Ohki [19] have shown that 5,6-acetylepimino-hexofuranose derivatives undergo ring-opening to the 5-acetamido-6-*O*-acetyl compounds in hot glacial acetic acid.

Addition of 2 equiv. of acetic anhydride to a suspension of the free 6,7-epimine (V) in methanol resulted in rapid dissolution of the solid. T.l.c., run immediately, showed the absence of starting material, and the formation of a single new material of lower polarity. Removal of the solvent gave a syrup, which showed no 1700 cm^{-1} absorption characteristic [19,20] of an *N*-acetylepimine, but the presence of amide I and amide II bands at 1650 and 1520 cm^{-1} demonstrated that both *N*-acetylation and ring-opening had occurred. The n.m.r. spectrum revealed the presence of an amide hydrogen atom [δ ($CDCl_3$) 6·25 (1H, d)] and a methoxy-group [δ ($CDCl_3$) 2·3 (3H, s)], proving the participation of methanol in the reaction. Furthermore, the material gave a molecular ion in the mass spectrum at *m/e* 309, and a peak at $M^+ - 59$, corresponding to the loss of MeCHOMe and the generation of the ion (VII). Therefore, *N*-acetylation of the epimine had occurred, followed by extremely rapid ring-opening by nucleophilic attack of methanol at C-7.

The chromatographically pure methoxy-amide could not be induced to crystallise; acetylation in pyridine–acetic anhydride gave a crystalline product, shown by t.l.c. to be a mixture of two materials, which was separated by counter-current distribution. The less polar,

[8] O. C. Dermer and G. E. Ham, 'Ethylenimine and other Aziridines: Chemistry and Applications,' Academic Press, New York, 1969, p. 230.
[9] H. Goldwhite, in 'Rodd's Chemistry of Carbon Compounds,' ed. S. Coffey, 2nd edn., Elsevier, New York, 1965, vol. 1B, ch. 5, p. 76.
[10] R. G. Pearson, H. Sobel, and J. Songstad, *J. Amer. Chem. Soc.*, 1968, **90**, 319.
[11] D. S. Tarbell and P. Noble, jun., *J. Amer. Chem. Soc.*, 1950, **72**, 2657.
[12] R. D. Guthrie and D. Murphy, *J. Chem. Soc.*, 1965, 3828.
[13] W. Meyer zu Reckendorf, *Chem. Ber.*, 1964, **97**, 325.
[14] J. Cleophax, S. D. Gero, and J. Hildesheim, *Chem. Comm.*, 1968, 94.
[15] D. H. Buss, L. Hough, and A. C. Richardson, *J. Chem. Soc.*, 1965, 2736.
[16] H. W. Heine, M. E. Fetter, and E. M. Nicholson, *J. Amer. Chem. Soc.*, 1959, **81**, 2202.
[17] H. C. Brown and A. Tsukamoto, *J. Amer. Chem. Soc.*, 1961, **83**, 4549.
[18] Y. Ali, A. C. Richardson, C. F. Gibbs, and L. Hough, *Carbohydrate Res.*, 1968, **7**, 255.
[19] H. Saeki and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 2471.
[20] H. L. Spell, *Analyt. Chem.*, 1967, **39**, 185.

major, product (*A*) was a tetra-acetate, as expected; the minor product (*B*) was found to be a monohydroxy-triacetate by elemental analysis and i.r. and mass spectra (M^+ **393**). Prolonged acetylation under the same conditions converted (*B*) into (*A*). This tetra-acetate was distinguished readily (m.p., i.r., n.m.r., and t.l.c. behaviour) from the (7*R*)-7-*O*-methyl tetra-acetate,[1] obtained from methyl thiolincosaminide. Since the stereochemistry of the 6,7-epimine is known,[6] and the methoxy-group is introduced at C-7, the tetra-acetate (A) must be the (7*S*)-7-*O*-methyl derivative (VIII) and the original methoxy-amide has structure (IX).

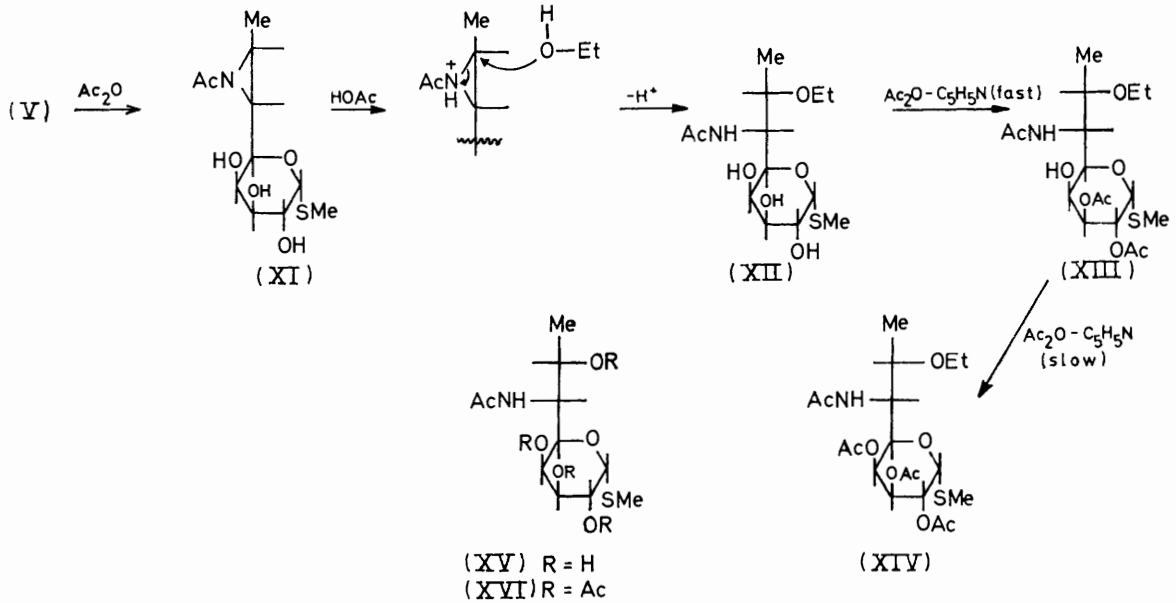


(VII) (VIII) $R^1 = R^2 = Ac$
(IX) $R^1 = R^2 = H$
(X) $R^1 = Ac, R^2 = H$

The n.m.r. spectrum of the triacetate (*B*) could be analysed completely; relative to the tetra-acetate (*A*), the C-4 proton quartet showed a diamagnetic shift of 1·5 p.p.m., thereby placing the unacetylated hydroxy-group at C-4, permitting the assignment of structure (X) to (*B*). Although the C-4 hydroxy-group is expected to be hindered sterically,[3,21] owing both to its axial orientation and to the bulk of the C-5 substituent, acetylation was unexpectely slower than in the case of the corresponding 7(*R*)-*O*-methyl series.[1] Examination of space-filling models indicates different preferred conformations for the methyl (7*S*)- and (7*R*)-*N*-acetyl-7-*O*-methyl-thiolincosaminides. In the 7*R*-case, this conformation involves hydrogen-bonding between the amide carbonyl and the C-4 hydroxy-groups, placing the methoxy-group some distance away from the 4-hydroxy-group. In the 7*S*-case, the conformation involves hydrogen-bonding between the amide hydrogen atom and both the ring oxygen and the 7-methoxy-oxygen atoms, placing the methyl group of the 7-methoxy-substituent in a position which would impose hindrance to the approach of the acylating agent to the 4-hydroxy-group. No such hindrance appears to be imposed by a 7(*S*)-hydroxy-group.

Treatment of a suspension of the epimine (V) in ethanol with acetic anhydride at room temperature resulted, as in the methanol reaction, in the immediate dissolution of the solid, but a crystalline solid separated within minutes. After 1 h, the absence of starting epimine was demonstrated by t.l.c., together with the formation of two poorly separated materials of lower polarity. The crystalline solid (*C*) corresponded (t.l.c.) to the material of higher R_F, and the filtrate showed a mixture of both substances, mainly that of lower R_F. After warming for 1 h, the filtrate showed the presence of the material of lower R_F only. Removal of the solvent gave a viscous syrup (*D*).

The i.r. spectrum of the solid (*C*) showed absorption at 1710 cm^{-1}, characteristic of an *N*-acetylepimine, and structure (XI) was confirmed by n.m.r. and mass spectra (M^+ 277), and elemental analysis. The i.r. spectrum of the syrup (*D*) showed peaks at 1640 (amide I) and 1505 cm^{-1} (amide II), proving that ring-opening had occurred; mass spectral peaks at *m/e* 323 (M^+) and 250 [the ion (VII)] demonstrated the participation of the ethanol in the reaction, and the presence of the ethoxy-group at C-7. Acetylation in pyridine–acetic anhydride, as in



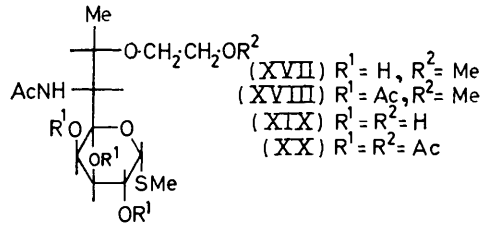
SCHEME 1

[21] J. M. Williams and A. C. Richardson, *Tetrahedron*, 1967, **23**, 1369; 1641.

the methoxy-series, gave a mixture, separated by counter-current distribution, of a tetra-acetate and a tri-acetate (assigned the 4-hydroxy-structure on a basis similar to that employed in the methoxy-series). These products are thus the ethoxy-amide (*D*) (XII) the tri-acetate (XIII), and the tetra-acetate (XIV). Whereas the filtrate from the acetylation of the epimine in ethanol, which contained both the *N*-acetylepimine (XI) and the ethoxy-amide (XII), contained only the ethoxy-amide after warming, the *N*-acetylepimine (XI) was stable to boiling ethanol. However, addition of glacial acetic acid to an ethanolic solution of (XI) resulted in ring-opening to give the ethoxy-amide (XII), slowly at room temperature but rapidly on gentle warming. The reactions involved, therefore, must be as illustrated (Scheme 1).

Treatment of the epimine (V) in water (in which it is soluble) with acetic anhydride at room temperature resulted in rapid ring-opening to give the crystalline hydroxy-amide (XV). Acetylation in pyridine–acetic anhydride gave the penta-acetate (XVI), with no indication of the hindrance to esterification of the 4-hydroxy-group observed with the 7-alkoxy-compounds. The penta-acetate (XVI) was distinguished readily (m.p., t.l.c.) from methyl *N*-acetyl-2,3,4,7-tetra-*O*-acetyl-1-thio-α-lincosaminide.[2,7] The cleavage pattern in the mass spectrum did not permit the assignment of the hydroxy-group to C-7, but this assignment was confirmed by the observation of the base-peak ion (XXXV) after conversion into the free amino-sugar. The reaction of acetic anhydride at room temperature with a suspension of the epimine (V) in isopropyl, butyl, or cyclohexyl alcohol gave the *N*-acetylepimine (XI) as the sole product. Use of 2-methoxyethanol as solvent gave the same result, but the *N*-acetylepimine underwent ring-opening in this solvent under gentle reflux in the presence of glacial acetic acid to give the syrupy methoxyethoxy-amide (XVII), which was converted into the crystalline tri-*O*-acetyl derivative (XVIII) in pyridine–acetic anhydride at 100°. Reflecting the greater acidity of ethylene glycol than of 2-methoxyethanol, acetylation of the epimine in ethylene glycol at room temperature gave the hydroxyethoxy-amide (XIX), which gave the crystalline tetra-*O*-acetyl derivative (XX) on acetylation at 100°.

Of the *N*-acetylepimino-sugars described in the literature, several have been obtained by reaction in methanol or ethanol with acetic anhydride, *e.g.* those of 2,3-epimino-D-manno- and -allo-pyranosides,[18,22] a 3,4-epimino-L-galactopyranoside,[23] * 5,6-epimino-L-altro- and ido-furanosides,[19] 5,6-epimino-D-gluco-[24] and -L-gulo-furanosides,[25] and 6,7-epimino-D- and -L-*glycero*-D-*galacto*-heptopyranosides;[26] in none of these cases was ring-opening observed. Barford and Richardson have described two epimino-glycitol derivatives, a 5,6-epimino-L-iditol[27] and a 3,4-epimino-D-iditol.[28] Although both



gave *N*-acetylepimines on reaction with acetic anhydride in ethanol, the former gave the ring-opened 5-acetamido-6-chloro-derivative on attempted acetylation with acetyl chloride in pyridine.

No explanation is suggested for the remarkable ease with which ring-opening accompanies *N*-acetylation in this 6,7-epimine. Under these conditions, no evidence of ring-opening by nucleophilic attack of acetate ion was found, in contrast to the acylation of simple epimines with acid anhydrides under mild conditions in inert solvents, which gives acyloxy-amides.[29,30]

Since the alkoxy-amides obtained by the ring-opening of the *N*-acetylepimine were not crystalline, and since *O*-acetylation for the characterisation of the products was complicated by the greatly reduced reactivity of the C-4 hydroxy-group, it was decided to examine the ring-opening of the fully acetylated epimine (XXI), which was obtained readily from the free epimine in pyridine–acetic anhydride. As with the *N*-acetylepimine (XI), the tetra-acetylepimine was stable to heating in methanol. While no reaction occurred at room temperature in methanol in the presence of glacial acetic acid, ring-opening occurred on heating to give the 7(*S*)-*O*-methyl tetra-acetate (VIII) in quantitative yield. Similarly, ring-opening occurred under the same conditions in isopropyl, propyl, and cyclohexyl alcohols to give the 7(*S*)-*O*-isopropyl (XXII), -propyl (XXIII), and -cyclohexyl (XXIV) derivatives. However, in the case of the less reactive alcohols, competition occurred in the nucleophilic attack at C-7 of the protonated acetylepimine between the alcohol and acetate ion; the penta-acetate formed was shown to be that (XVI) obtained from the aqueous acetylation of the free epimine followed by *O*-acetylation.

From the reaction between the tetra-acetylepimine and

* H. H. Baer and T. Neilson (*Canad. J. Chem.*, 1965, **43**, 840) describe a solvolysis product of methyl 3-acetamido-2,3-dideoxy-4,6-di-*O*-methylsulphonyl-β-D-*arabino*-hexopyranoside as being either a 3,4-acetylepimine or a 3,6-acetylpyrrolidine. The i.r. spectrum [no amide II, amide I at 1643 (mull) or 1660 (CCl_4) cm^{-1}] seems to rule out the acetylepimine structure.[19]

[22] D. H. Buss, L. Hough, and A. C. Richardson, *J. Chem. Soc.*, 1963, 5295.

[23] A. D. Barford and A. C. Richardson, *Carbohydrate Res.*, 1967, **4**, 408.

[24] H. Saeki and E. Ohki, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 2477.

[25] J. S. Brimacombe, F. Hunedy, and M. Stacey, *Carbohydrate Res.*, 1970, **13**, 447.

[26] H. Saeki and E. Ohki, *Chem. and Pharm. Bull.* (*Japan*), 1970, **18**, 789.

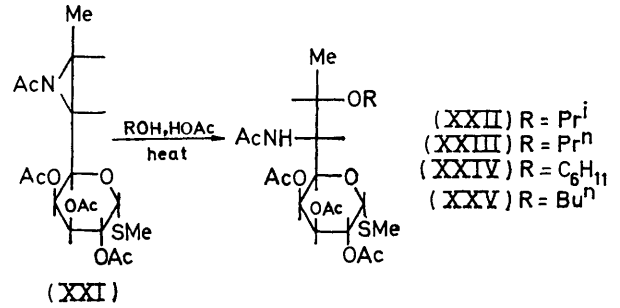
[27] A. D. Barford and A. C. Richardson, *Carbohydrate Res.*, 1970, **14**, 217.

[28] A. D. Barford and A. C. Richardson, *Carbohydrate Res.*, 1970, **14**, 231.

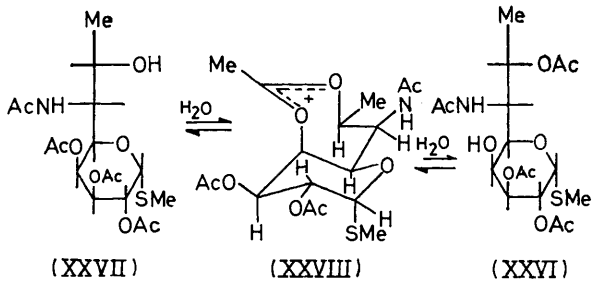
[29] P. Loewrigkeit, G. T. Del Franco, N. Georgalas, and P. Resnick, *J. Org. Chem.*, 1968, **33**, 3344.

[30] E. Testa, L. Fontanella, and V. Aresi, *Annalen*, 1964, **676**, 151.

n-butyl alcohol, three products were isolated by countercurrent distribution. The product of lowest polarity (K * 5·42) was the expected 7(*S*)-*O*-butyl tetra-acetate (XXV), formed in 22% yield, and the penta-acetate [(XVI), K 1·22] was produced in similar yield. The

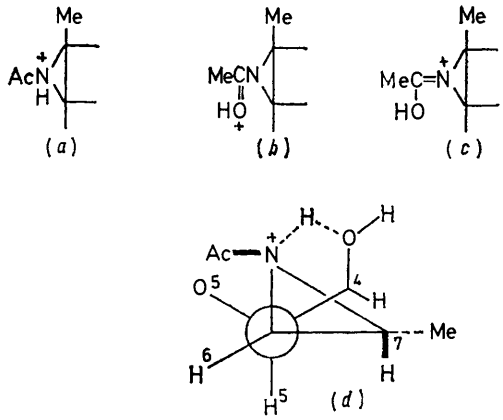


major peak (K 0·53) was shown to contain a mixture of two materials (t.l.c.) and was separated into the minor (*E*) and major (*F*) components by chromatography. Both showed amide I and II absorptions of ring-opened products and, in their mass spectra, both showed M^+ 421, corresponding to monohydroxy-tetra-acetates. Acetylation of each in pyridine–acetic anhydride gave the same penta-acetate (XVI). Their n.m.r. spectra allowed complete analysis. The spectrum of component (*E*), as compared with the penta-acetate, showed a diamagnetic shift of the C-4 proton signal of 1·50 p.p.m., permitting the assignment of the hydroxy-group to C-4 (XXVI); that of component (*F*) showed a diamagnetic shift of 1·08 p.p.m. of the C-7 proton signal relative to the penta-acetate (XVI), showing (*F*) to be the 7-hydroxy-tetra-acetate (XXVII). Molecular models show that a seven-membered acetoxonium ion (XXVIII) can be formed with ease. The sample of butyl alcohol was found by Karl Fischer titration to contain 0·23% of water (w/v), and the reaction must have involved ring-opening by water to give the 7-hydroxy-compound (XXVII), equilibrated with the 4-hydroxy-compound (XXVI) *via* the 4,7-ion (XXVIII). It is of interest that the combined hydroxy-tetra-acetates [(XXVI) and (XXVII)] were formed in greater yield (56%) than the penta-acetate [(XVI), 22%], in spite of the excess of acetic acid present (molar ratio acetic acid : water, 16·8 : 1), and must reflect the greater nucleophilicity of water than of acetic acid or acetate ion.



Apart from the competitive attack of acetic acid (or water, if present) with the tetra-acetylepimine, no other mode of opening of the acetyl-epimino-ring was observed. That ring-opening occurs exclusively by attack of the reagent at C-7 is in conformity with the well established unfavourable interactions involving S_N2-displacements at C-6 occasioned by *galacto*-stereochemistry.[31] Also, there was no indication of *cis*-ring-opening, which might result from S_N1 heterolysis of the N–C(7) bond. Richardson and his co-workers [18] have considered the possibility of the anomalous *trans*-diequatorial opening of carbohydrate 2,3-benzoylepimines being due to an S_N1 process.

The greater reactivity toward ring opening with methanol–acetic acid of the *N*-acetylepimine than of the fully acetylated epimine, as reflected in the rapid opening of the former at room temperature whereas the latter required heating, was surprising. In view of the results of Brown and Tsukamoto,[17] the structure of the protonated *N*-acetylepimino-group must be (*a*) or (*b*), and cannot be (*c*). The protonation of the weakly basic *N*-acetylepimino-group by the weakly acidic acetic acid must be an equilibrium reaction in which the concentration of the protonated form is very low. Examination of molecular models indicates that hydrogen-bonding between the 4-hydroxy-group and the proton in structure (*a*) can occur, as illustrated in the Newman projection (*d*), viewed along the bond from C-6 to C-5. Such



hydrogen bonding would stabilise the protonated *N*-acetylepimino-group, and thus lead to a higher concentration of the protonated species in the equilibrium mixture and, therefore, to a greater rate of reaction if the 4-hydroxy-group is present than if it is esterified.

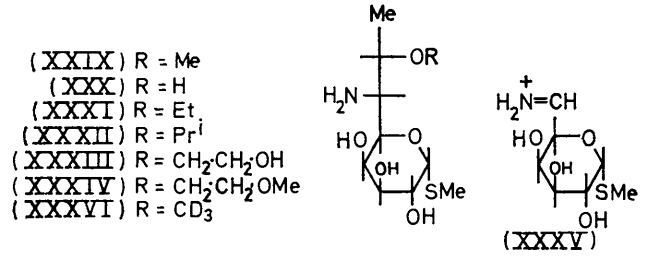
Hydrazinolysis of the 7-*O*-methyl tetra-acetate (VIII) gave crystalline methyl (7*S*)-7-deoxy-7-methoxy-1-thio-α-lincosaminide (XXIX), which was not distinguished by t.l.c. from material in a minor t.l.c. zone of the reaction mixture from the free epimine and methanol in the presence of sulphuric acid. The crystalline (7*S*)-7-deoxy-7-hydroxy (XXX), -ethoxy (XXXI), -isopropoxy (XXXII), -(2-hydroxyethoxy) (XXXIII), and -(2-methoxyethoxy) (XXIV) free sugars were obtained similarly from the fully acetylated derivatives. In their mass spectra, all showed molecular ions, and all showed a base

* See ref. 2 for a definition of K.

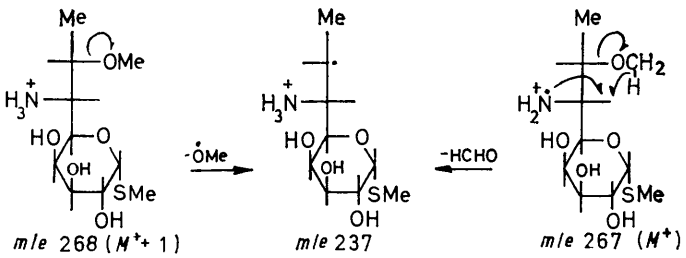
[31] D. H. Ball and F. W. Parrish, *Adv. Carbohydrate Chem. and Biochem.*, 1969, **24**, 139.

peak at *m/e* 208, due to cleavage between C-6 and C-7 with the formation of the stable iminium ion (XXXV).

All the free sugars also showed a significant peak at *m/e* 237, resulting from the cleavage of the C(7)–OR bond. Illustrated (Scheme 2) in the case of the 7-methoxy-sugar, this ion could have arisen by the concomitant

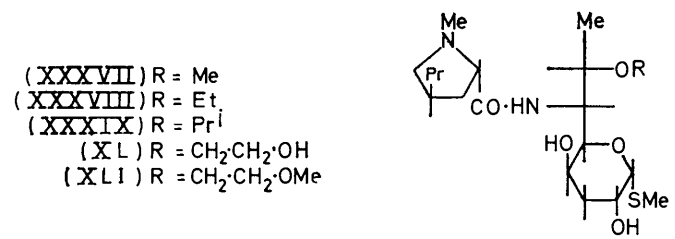


transfer of a hydrogen atom from the methoxy-group to the amine function with the loss of formaldehyde or, since a fairly strong $(M^+ + 1)$ ion was observed, it could have arisen by the more conventional loss of MeO· from that ion. This question was resolved by means of deuterium labelling. The tetra-acetylepimine (XXI) reacted with [$^2H_4$]methanol in the presence of acetic acid to give the 7-[$^2H_3$]methoxy-tetra-acetate which, on hydrolysis, gave the free [$^2H_3$]methoxy-amino-sugar (XXXVI). The mass spectrum of (XXXVI) showed *m/e* 270 (M^+), a small peak at *m/e* 271 ($M^+ + 1$), and a significant ion at *m/e* 238. This *m/e* 238 peak can be accounted for only by the concomitant transfer of a deuterium atom and the loss of [$^2H_2$]formaldehyde, since the loss of the [$^2H_3$]methoxy-group from *m/e* 271 ($M^+ + 1$) would result in a fragment of *m/e* 237.



SCHEME 2

Condensation of the 7-*O*-methyl sugar (XXIX) with 1-methyl-*trans*-4-propyl-L-proline *via* its mixed anhydride [7] from isobutyl chloroformate gave (7*S*)-7-deoxy-7-methoxylincomycin (XXXVII), isolated as its amorphous hydrochloride. By the same method, the other



free amino-sugars were converted into the (7*S*)-ethoxy- (XXXVIII), -isopropoxy- (XXXIX), -(2-hydroxyethoxy)- (XL), and -(2-methoxyethoxy)- (XLI) analogues of lincomycin, isolated also as their amorphous hydrochlorides.

Antibacterial Activities.—The (7*S*)-7-methoxy-analogue (XXXVII) shows 3·5 times the response of lincomycin against *Sarcina lutea* in the standard curve agar diffusion assay; [32a] broth dilution assay shows a two- to four-fold enhancement of activity against a variety of Gram-positive bacteria with no broadening of the spectrum of lincomycin. In the mouse infected experimentally with a lethal challenge of *Staphyloccus aureus*,[32b] this analogue shows a CD_{50} not distinguished from that of (7*S*)-7-chloro-7-deoxy-lincomycin.[4]

The tabulated results show that, while replacement of the 7-hydroxy-group of 7-*epi*-lincomycin (II) by a methoxy-group results in a significant enhancement of antibacterial activity, replacement by larger alkoxy- and substituted alkoxy-groups results in smaller enhancement (by the ethoxy- and isopropoxy-substituents) or in decreased activity (by the 2-hydroxyethoxy- and 2-methoxyethoxy-substituents).

Standard curve assay of analogues *vs. S. lutea*

7(*S*)-Substituent	Formula	Relative activity (lincomycin = 1·0)
OH	(II)	0·5
OMe	(XXXVII)	3·5
OEt	(XXXVIII)	1·1
OPr^i	(XXXIX)	0·7
O·CH_2·CH_2·OH	(XL)	0·3
O·CH_2·CH_2·OMe	(XLI)	0·3

EXPERIMENTAL

M.p.s were determined with a Thomas Hoover 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,[33] 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 rotary 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 at 60 MHz with a Varian A60A spectrometer for solutions in $CDCl_3$ with Me_4Si as internal standard. For the purposes of this work, spectra of solutions in D_2O were calibrated against the HOD peak at δ 4·67. Mass spectra were recorded with an Atlas CH-4 spectrometer (direct inlet) at 70 eV.

Methyl (7S)-N-*Acetyl*-7-*deoxy*-7-*methoxy*-1-*thio*-α-*lincosaminide* (*Methyl* 6-*Acetylamino*-6,7,8-*trideoxy*-7-*methoxy*-1-*thio*-L-threo-α-D-galacto-*octopyranoside*) (IX).—Acetic anhydride (2·04 g, 20 mmol) was added to a stirred suspension of methyl (6*R*,7*R*)-6-deamino-7-deoxy-6,7-epimino-1-thio-α-lincosaminide [6] (V) (2·35 g, 10 mmol) at room temperature. The solid dissolved at once, and t.l.c. (methanol–chloroform, 1 : 1) showed the absence of epimine (R_F 0·48) and the presence of a single new zone (R_F 0·69). Removal of solvent gave a syrup containing acetic acid, which was removed

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

[32] (*a*) L. J. Hanka, D. J. Mason, M. R. Burch, and R. W. Treick, *Antimicrobial Agents and Chemotherapy*, 1962, p. 565; (*b*) C. Lewis, H. W. Clapp, and J. E. Grady, *ibid.*, p. 570.

by chromatography [methanol–chloroform (1 : 10)]. The syrupy product (3·05 g) could not be induced to crystallise; $\nu_{max.}$ (syrup) 1650 (amide I) and 1520 cm^{-1} (amide II), δ ($CHCl_3$) 6·25 (1H, d, amide) and 2·3 (3H, s, OMe), *m/e* 309 (M^+) and 250 [M^+ − 59, the ion (VII)].

Methyl (7S)-N-*Acetyl*-2,3,4-*tri*-O-*acetyl*-7-*deoxy*-7-*methoxy*-1-*thio*-α-*lincosaminide* (VIII) *and Methyl* (7S)-N-*Acetyl*-2,3-*di*-O-*acetyl*-7-*deoxy*-7-*methoxy*-1-*thio*-α-*lincosaminide* (X).—Treatment of the syrupy methoxy-amide (IX) (2·90 g) in pyridine with acetic anhydride at room temperature for 24 h and isolation of the product by standard procedures gave a solid (4·06 g) which separated from ethyl acetate–Skellysolve B * as needles, m.p. 245—247°. Both the solid and the mother liquors showed only one zone on t.l.c. (R_F 0·14) in acetone–Skellysolve B (1 : 2·5), but both showed two zones (R_F 0·29 and 0·37) in ethyl acetate–hexane (1 : 1; double development). Counter-current distribution in ethanol–water–ethyl acetate–cyclohexane (1 : 1 : 1 : 1) gave two peaks (K 0·59 and 1·14) after 500 transfers. The faster-moving component (*A*) was isolated as a solid (2·72 g) which separated from ethyl acetate–Skellysolve B as *needles* of the tetra-acetate (VIII), m.p. 235—236° [depressed on admixture with the 7(*R*)-methoxy-compound,[1] m.p. 212—213°], $[\alpha]_D$ +205° (*c* 0·99 in $CHCl_3$), *m/e* 435 (M^+), 420 (M^+ − Me), and 388 (M^+ − SMe), $\nu_{max.}$ 1750 (ester), 1650 (amide I), and 1560—1545sh and 1535 cm^{-1} (amide II) (Found: C, 49·8; H, 6·9; N, 3·65; S, 7·9; OMe, 7·1. $C_{18}H_{29}NO_9S$ requires C, 49·6; H, 6·7; N, 3·2; S, 7·4; OMe, 7·4%).

The slower-moving component (*B*), isolated as a solid (1·24 g), was shown to be the 2,3-di-*O*-acetyl-4-hydroxy-derivative (X); it separated from ethyl acetate–Skellysolve B as *prisms*, m.p. 189—190°, $[\alpha]_D$ +275° (*c* 1·02 in $CHCl_3$), *m/e* 393 (M^+), 375 (M^+ − H_2O), and 346 (M^+ − SMe), $\nu_{max.}$ 1750 and 1735 (ester), 1660 (amide I), and 1530 cm^{-1} (amide II) (Found: C, 48·7; H, 7·1; N, 3·9; S, 8·0; OMe, 8·0. $C_{16}H_{27}NO_8S$ requires C, 48·8; H, 6·9; N, 3·6; S, 8·15; OMe, 7·9%).

Acetylation of (X) occurred slowly in pyridine–acetic anhydride. It could be followed by t.l.c. in ethyl acetate–hexane (1 : 1), and was complete in 4 days; the tetra-acetate (VIII) was identified by t.l.c., m.p., and mixed m.p.

Methyl (6R,7R)-6,7-*Acetylepimino*-6-*deamino*-7-*deoxy*-1-*thio*-α-*lincosaminide* (*Methyl* 6,7-*Acetylepimino*-6,7,8-*trideoxy*-1-*thio*-D-erythro-α-D-galacto-*octopyranoside*) (XI) *and Methyl* (7S)-N-*Acetyl*-7-*deoxy*-7-*ethoxy*-1-*thio*-α-*lincosaminide* (XII).—Treatment of a stirred suspension of the epimine (V) (2·35 g, 10 mmol) in ethanol (25 ml) at room temperature with acetic anhydride (2·04 g, 20 mmol) led to the rapid dissolution of the solid, followed almost at once by the separation of a crystalline solid. After 1 h, t.l.c. in methanol–chloroform (1 : 5) showed the absence of starting material (R_F 0·34) and the presence of two new zones (R_F 0·49 and 0·60). The solid (*C*) was filtered off and washed with ethanol. It showed only the R_F 0·60 zone on t.l.c., and was the 6,7-acetylepimine (XI), m.p. 145—146°, unchanged by recrystallisation from ethanol, from which it separated in *platelets*, $[\alpha]_D$ +253° (*c* 0·79 in H_2O), $\nu_{max.}$ 1710 cm^{-1} (amide I), *m/e* 277 (M^+), 230 (M^+ − SMe), and 188 (230 − $H_2C{=}C{=}O$) (Found: C, 47·6; H, 6·7; N, 5·2; S, 11·3. $C_{11}H_{19}NO_5S$ requires C, 47·6; H, 6·9; N, 5·05; S, 11·7%).

When the acetylation of the epimine was conducted in isopropyl, butyl, or cyclohexyl alcohol, the sole product was the 6,7-acetylepimine (XI), isolated in almost quantitative yield.

T.l.c. of the filtrate from the acetylation in ethanol showed a minor zone (R_F 0·60) and a major zone (R_F 0·49). After 1 h under reflux, only the material corresponding to the lower zone remained. Removal of the solvent gave a syrup containing acetic acid; chromatography in methanol–chloroform (1 : 12) gave a syrup (*D*) (1·17 g), which was not obtained crystalline, showing $\nu_{max.}$ (syrup) 1640 (amide I) and 1505 cm^{-1} (amide II), *m/e* 323 (M^+) and 250 [the ion (VII)].

Methyl (7S)-N-*Acetyl*-2,3-*di*-O-*acetyl*-7-*deoxy*-7-*ethoxy*-1-*thio*-α-*lincosaminide* (XIII) *and Methyl* (7S)-N-*Acetyl*-2,3,4-*tri*-O-*acetyl*-7-*deoxy*-7-*ethoxy*-1-*thio*-α-*lincosaminide* (XIV).—Acetylation of the syrupy ethoxy-amide (XII) (3·57 g), as for the methoxy-compound (IX), gave a solid (4·99 g) showing two zones on t.l.c. (ethyl acetate–hexane, 1 : 1; double development) of R_F 0·31 and 0·37. Counter-current distribution in ethanol–water–ethyl acetate–cyclohexane (1 : 1 : 1 : 1) gave, after 500 transfers, peaks at K 0·87 and 1·59. The faster-moving component was obtained as needles (2·15 g) from ethyl acetate–Skellysolve B of the *ethoxy-tetra-acetate* (XIV), m.p. 254—255°, $[\alpha]_D$ +199° (*c* 0·86 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1550 cm^{-1} (amide II), *m/e* 449 (M^+), 402 (M^+ − SMe), and 389 (M^+ − HOAc) (Found: C, 50·75; H, 7·1; N, 3·3; S, 7·3 OEt, 10·25. $C_{19}H_{31}NO_9S$ requires C, 50·8; H, 6·95; N, 3·1; S, 7·1; OEt, 10·0%).

The slower-moving component, the 2,3-di-*O*-acetyl-4-hydroxy-compound (XIII), separated from ethyl acetate in *prisms* (1·73 g), m.p. 215·5—216·5° with a change of crystal structure at 202°, $[\alpha]_D$ +261° (*c* 1·05 in $CHCl_3$), $\nu_{max.}$ 1750, 1725 (ester), 1660 (amide I), and 1525 cm^{-1} (amide II), *m/e* 408 (M^+ + 1), 407 (M^+), 389 (M^+ − H_2O), 360 (M^+ − SMe), and 347 (M^+ − HOAc) (Found: C, 50·2; H, 7·3; N, 3·5; S, 7·6. $C_{17}H_{29}NO_8S$ requires C, 50·1; H, 7·2; N, 3·4; S, 7·9%). This di-*O*-acetyl derivative was converted into the tri-*O*-acetate (XIV) slowly at room temperature by prolonged acetylation.

Methyl N-*Acetyl*-7-epi-1-*thio*-α-*lincosaminide* (*Methyl* 6-*Acetylamino*-6,8-*dideoxy*-1-*thio*-L-threo-α-D-galacto-*octopyranoside*) (XV) *and its* 2,3,4,7-*Tetra*-O-*acetyl Derivative* (XVI).—Acetic anhydride (2·04 g, 20 mmol) was added to a solution of the epimine (V) (2·35 g, 10 mmol) in water (25 ml); after 1 h, t.l.c. in methanol–chloroform (1 : 1) showed the absence of epimine (R_F 0·54) and the formation of material of R_F 0·62. Removal of solvent gave a syrup which crystallised from methanol to give the *N*-acetyl-7-*epi*-compound (XV) as stout *rods* (2·3 g), m.p. 218—219° (depressed sharply on admixture with methyl *N*-acetyl-1-thio-α-lincosaminide,[7] m.p. 243—245°), $[\alpha]_D$ +260° (*c* 1·03 in H_2O), $\nu_{max.}$ 1650 (amide I) and 1525 cm^{-1} (amide II), *m/e* 295 (M^+), 248 (M^+ − SMe), 230 (M^+ − H_2O), and 212 (230 − H_2O) (Found: C, 44·9; H, 7·0; N, 5·0; S, 10·6. $C_{11}H_{21}NO_6S$ requires C, 44·7; H, 7·2; N, 4·7; S, 10·9%).

Acetylation of (XV) in pyridine–acetic anhydride overnight at room temperature gave the tetra-*O*-acetate (XVI), which separated from ethyl acetate as *needles*. m.p. 312—313° (depressed sharply on admixture with methyl *N*-acetyl-tetra-*O*-acetyl-1-thio-α-lincosaminide,[7] double m.p. 210—212° and 218—220°), $[\alpha]_D$ +182° (*c* 0·59 in $CHCl_3$), $\nu_{max.}$ 1750 and 1670sh (ester), 1655 (amide I), and 1560 and 1545 cm^{-1} (amide II), *m/e* 463 (M^+), 416 (M^+ − SMe), and 403 (M^+ − HOAc) (Found: C, 49·2; H, 6·5; N, 3·1; S, 6·8. $C_{19}H_{29}NO_{10}S$ requires C, 49·2; H, 6·3; N, 3·0; S, 6·9%).

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

Methyl (7S)-N-*Acetyl*-7-*deoxy*-7-(2-*hydroxyethoxy*)-1-*thio*-α-*lincosaminide* (XIX) *and its* 2,2',3,4-*Tetra*-O-*acetyl Derivative* (XX).—Acetylation of a suspension of the epimine (V) (5·0 g) in ethylene glycol (50 ml) with acetic anhydride under the usual conditions gave the hydroxyethoxy-amide (XIX) as a syrup, R_F 0·32 in methanol–chloroform (1 : 5), $\nu_{max.}$ (syrup) 1640 and 1545 cm^{-1} (amide I and II). Acetylation of this syrup in pyridine–acetic anhydride at 100° for 8 h and isolation by conventional means gave a solid (10·59 g) showing one zone only on t.l.c. in ethyl acetate–hexane–methanol (10 : 10 : 0·4); crystallisation from ethyl acetate gave the tetra-*O*-acetate (XX) as *needles*. m.p. 223—225°, $[\alpha]_D$ +172° (*c* 1·01 in $CHCl_3$), $\nu_{max.}$ 1750 (ester), 1650 (amide I), and 1565 cm^{-1} (amide II), *m/e* 507 (M^+), 492 (M^+ — Me), 464 (M^+ — Ac), 460 (M^+ — SMe), 447 (M^+ — HOAc), and 87 ($CH_2CH_2{}^+\dot{O}Ac$) (Found: C, 49·6; H, 6·6; N, 2·9; S, 6·6. $C_{21}H_{33}NO_{11}S$ requires C, 49·7; H, 6·55; N, 2·8; S, 6·3%).

Methyl (7S)-N-*Acetyl*-7-*deoxy*-7-(2-*methoxyethoxy*)-1-*thio*-α-*lincosaminide* (XVII) *and its* 2,3,4-*Tri*-O-*acetyl Derivative* (XVIII).—Acetylation of a suspension of the epimine (V) in 2-methoxyethanol with acetic anhydride at room temperature gave a quantitative yield of the *N*-acetylepimine (XI). Treatment of (XI) (5·9 g, 21·3 mmol) in 2-methoxyethanol (50 ml) and acetic acid (5·40 g, 106·5 mmol) under reflux overnight gave a colourless solution; t.l.c. showed the absence of acetylepimine (R_F 0·26) and a major zone, R_F 0·14, in ethyl acetate–methanol (10 : 0·5). Chromatography in methanol–chloroform (1 : 10) of the residue obtained on the removal of solvent gave (XVII) as a syrup (6·66 g), $\nu_{max.}$ (syrup) 1650 and 1510 cm^{-1}, δ 2·4 (3H, s, OMe). Acetylation in pyridine–acetic anhydride at 100° for 8 h, followed by standard isolation procedures, gave a tan solid (9·0 g) showing one zone only (R_F 0·19) on t.l.c. in ethyl acetate–hexane–methanol (10 : 10 : 0·4). This tri-*O*-acetate (XVIII) crystallised from ethyl acetate–Skellysolve B in *needles*, m.p. 222—223°, $[\alpha]_D$ +177° (c 1·08 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1545 cm^{-1} (amide II), *m/e* 479 (M^+), 464 (M^+ — Me), 447 (M^+ — MeOH), 432 (M^+ — SMe), and 419 (M^+ — HOAc) (Found: C, 50·1; H, 7·0; N, 2·8; S, 6·3; OMe, 6·3. $C_{20}H_{33}NO_{10}S$ requires C, 50·1; H, 6·9; N, 2·9; S, 6·7; OMe, 6·5%).

Methyl (6R,7R)-2,3,4-*Tri*-O-*acetyl*-6,7-*acetylepimino*-6-*deamino*-7-*deoxy*-1-*thio*-α-*lincosaminide* (*Methyl* 2,3,4-*Tri*-O-*acetyl*-6,7-*acetylepimino*-6,7,8-*trideoxy*-1-*thio*-D-erythro-α-D-galacto-*octopyranoside*) (XXI).—The epimine (V) (10·0 g) in pyridine (130 ml) and acetic anhydride (60 ml) was set aside for 20 h at room temperature; solvent was removed to give a crystalline solid. Recrystallisation from ethyl acetate gave the tetra-acetylepimine (XXI) as *prisms*, m.p. 173·5—175°, $[\alpha]_D$ +222° (*c* 0·91 in $CHCl_3$), $\nu_{max.}$ 1755 and 1740 (ester), and 1700 cm^{-1} (amide I), *m/e* 403 (M^+), 361 (M^+ — $H_2C{=}C{=}O$), 356 (M^+ — SMe), 344 (M^+ — AcO), 343 (M^+ — HOAc), and 314 (M^+ — SMe — $H_2C{=}C{=}O$) (Found: C, 50·4; H, 6·3; N, 3·4; S, 8·3. $C_{17}H_{25}NO_8S$ requires C, 50·6; H, 6·25; N, 3·5; S, 7·95%).

Reaction of the Tetra-acetylepimine (XXI) *with Methanol and Acetic Acid*.—The tetra-acetylepimine (5·0 g, 12·4 mmol) dissolved rapidly in a mixture of methanol (50 ml) and acetic acid (5·25 g, 86·8 mmol), and the solution was heated under gentle reflux for 6 h; t.l.c. (acetone–Skellysolve B, 1 : 1) showed the absence of starting material (R_F 0·59) and the formation of a single new material (R_F 0·46), not separated from the methoxy-tetra-acetate (VIII). Removal of the solvent gave a syrup, which was dissolved in methylene chloride; the solution was washed with saturated aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated to leave a chromatographically pure crystalline solid (5·31 g), which separated from ethyl acetate–Skellysolve B in needles, m.p. 235—236°, of the methoxy-tetra-acetate (VIII) (m.p., mixed m.p., and i.r. and n.m.r. spectra).

Methyl (7S)-N-*Acetyl*-2,3,4-*tri*-O-*acetyl*-7-*deoxy*-7-*isopropoxy*-1-*thio*-α-*lincosaminide* (XXII).—Under the same conditions as just described, but with isopropyl alcohol instead of methanol, a solid (5·14 g) was obtained from the tetra-acetylepimine (4·01 g). T.l.c. demonstrated the absence of starting material (R_F 0·68), and the presence of two products, R_F 0·59 and 0·52 in acetone–Skellysolve B (1 : 1). Counter-current distribution in ethanol–water–ethyl acetate–cyclohexane (1 : 1 : 1 : 1) gave, after 500 transfers, two peaks of *K* 0·82 and 2·29. Isolation of the slower moving component gave a crystalline solid (1·81 g), shown to be the 7(*S*)-acetate (XVI) (m.p., mixed m.p., and i.r.).

Isolation of the faster-moving component gave a solid (2·67 g) which separated from ethyl acetate in flattened needles, shown to be the 7(*S*)-*isopropoxy-tetra-acetate* (XXII), m.p. 253—254°, $[\alpha]_D$ +192° (*c* 0·54 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1550 cm^{-1} (amide II), *m/e* 463 (M^+), 416 (M^+ — SMe), and 404 (M^+ — OPr^i) (Found: C, 52·0; H, 7·1; N, 3·2; S, 6·6. $C_{20}H_{33}NO_9S$ requires C, 51·8; H, 7·2; N, 3·0; S, 6·9%).

Methyl (7S)-N-*Acetyl*-2,3,4-*tri*-O-*acetyl*-7-*deoxy*-7-*propoxy*-1-*thio*-α-*lincosaminide* (XXIII).—Analogously, from the tetra-acetylepimine (2·92 g) and propyl alcohol (25 ml), a crude solid (3·11 g) was obtained; chromatography in methanol–chloroform (1 : 10) gave the product (XXIII) (2·52 g) as *needles* from ethyl acetate–Skellysolve B, m.p. 241·5—242·5°, $[\alpha]_D$ +193° (*c* 0·93 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1550 cm^{-1} (amide II), *m/e* 463 (M^+), 416 (M^+ — SMe), and 404 (M^+ — OPr^n) (Found: C, 51·8; H, 7·0; N, 3·2; S, 6·8. $C_{20}H_{33}NO_9S$ requires C, 51·8; H, 7·2; N, 3·0; S, 6·9%).

Methyl (7S)-N-*Acetyl*-2,3,4-*tri*-O-*acetyl*-7-*cyclohexyloxy*-7-*deoxy*-1-*thio*-α-*lincosaminide* (XXIV) —The reaction of the tetra-acetylepimine (2·92 g) with cyclohexanol (25 ml) was conducted in an oil-bath at 100° for 8 h. The solution showed two products on t.l.c. in acetone–Skellysolve B (1 : 1) of R_F 0·34 and 0·54, separated by chromatography in ethyl acetate–Skellysolve B (3 : 1). The more polar product (1·1 g) was the 7(*S*)-acetoxy-compound (XVI); the less polar material (1·6 g) was the cyclohexyloxy-derivative (XXIV), which separated from ethyl acetate in flattened *needles*, m.p. 266—268°, $[\alpha]_D$ +163° (*c* 1·05 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1545 cm^{-1} (amide II), *m/e* 503 (M^+), 456 (M^+ — SMe), and 444 (M^+ — AcO) (Found: C, 54·9; H, 7·5; N, 2·9; S, 6·65. $C_{23}H_{37}NO_9S$ requires C, 54·85; H, 7·4; N, 2·8; S, 6·4%).

Methyl (7S)-N-*Acetyl*-2,3,4-*tri*-O-*acetyl*-7-*butoxy*-7-*deoxy*-1-*thio*-α-*lincosaminide* (XXV) *and Methyl* N-*Acetyl*-2,3,4- *and* 2,3,7-*tri*-O-*acetyl*-1-*thio*-α-*lincosaminides* [(XXVII) *and* (XXVI)].—The reaction of the tetra-acetylepimine (10·0 g) in butanol (100 ml) was carried out in an oil-bath at 100° for 12 h, and was shown by t.l.c. to give several products. The crude product (10·99 g) was subjected to counter-current distribution (ethanol–water–ethyl acetate–cyclohexane, 1 : 1 : 1 : 0·75); after 500 transfers, peaks of *K* 0·53, 1·22, and 5·42 were found. The material of *K* 1·22 was shown to be the 7(*S*)-penta-acetate (2·49 g, 22%). The least polar product, the 7(*S*)-butoxy-tetra-acetate (2·60 g, 22%; R_F 0·46 in acetone–Skellysolve B, 1 : 1) crystallised from

ethyl acetate in *needles*, m.p. 235·5—237°, $[\alpha]_D$ +182° (*c* 0·64 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1550 cm^{-1} (amide II), *m/e* 477 (M^+) and 430 (M^+ — SMe) (Found: C, 53·1; H, 7·2; N, 3·1; S, 6·65. $C_{21}H_{35}NO_9S$ requires C, 52·8; H, 7·4; N, 2·9; S, 6·7%).

The peak of *K* 0·53 fitted the theoretical curve well, but showed (t.l.c. in acetone–Skellysolve B, 1 : 1) a major and a minor zone (R_F 0·25 and 0·31), separated by chromatography (methanol–chloroform, 1 : 15; R_F 0·29 and 0·52). The minor component (1·20 g) separated as platelets from ethyl acetate, and was identified as the 4-*hydroxy-tetra-acetate* (XXVI), m.p. 194—195°, $[\alpha]_D$ +236° (*c* 0·99 in $CHCl_3$), $\nu_{max.}$ 1740 (ester), 1650 (amide I), and 1530 cm^{-1} (amide II), *m/e* 421 (M^+), 403 ($M^+ - H_2O$), and 374 (M^+ — SMe) (Found: C, 48·6; H, 6·5; N, 3·2; S, 7·7. $C_{17}H_{27}NO_9S$ requires C, 48·4; H, 6·5; N, 3·3; S, 7·6%). The second component (4·4 g), identified as the 7-*hydroxy-tetra-acetate* (XXVII), crystallised from methanol in needles, m.p. 187—188°, $[\alpha]_D$ +228° (*c* 0·83 in $CHCl_3$), $\nu_{max.}$ 1740, 1655, and 1540 cm^{-1} (ester, amide I, and amide II, respectively), *m/e* 421 (M^+) and 374 (M^+ — SMe) (Found: C, 48·5; H, 6·4; N, 3·1; S, 7·9%).

Acetylation of both (XXVI) and (XXVII) in pyridine–acetic anhydride overnight at room temperature yielded the (7*S*)-penta-acetate (XVI), m.p. 312—313° (m.p., mixed m.p., and i.r. comparison).

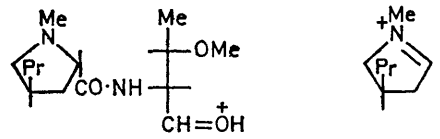
Methyl (7S)-7-*Deoxy*-7-*methoxy*-1-*thio*-α-*lincosaminide* (XXIX).—The methoxy-tetra-acetate (VIII) (4·08 g) in hydrazine hydrate (25 g) was heated overnight under gentle reflux. Volatile materials were distilled off at 100° and 15 mmHg to give a syrup (2·2 g); the amino-sugar separated from acetonitrile in *needles*, m.p. 154—155°, $[\alpha]_D$ +260° (*c* 0·56 in H_2O), *m/e* 268 ($M^+ + 1$), 267 (M^+), 249 ($M^+ - H_2O$), 237 ($M^+ - CH_2O$), 220 (M^+ — SMe), and 208 (M^+ — MeĊHOMe) (Found: C, 45·15; H, 8·0; N, 5·1; S, 12·2; OMe, 11·9. $C_{10}H_{21}NO_5S$ requires C, 44·9; H, 7·9; N, 5·2; S, 12·0; OMe, 11·6%).

By the foregoing procedure, the following amino-sugars were obtained from their corresponding acetates: *methyl* (7S)-7-*deoxy*-7-*hydroxy*-1-*thio*-α-*lincosaminide* (*methyl* 7-epi-1-*thio*-α-*lincosaminide*) (XXX), platelets from methanol, m.p. 211—212°, $[\alpha]_D$ +280° (*c* 0·77 in H_2O), *m/e* 253 (M^+), 238 (M^+ — Me), 208 (M^+ — MeĊHOH), and 206 (M^+ — SMe) (Found: C, 42·8; H, 7·7; N, 5·85; S, 12·7. $C_9H_{19}NO_5S$ requires C, 42·7; H, 7·6; N, 5·5; S, 12·7%); *methyl* (7S)-7-*deoxy*-7-*ethoxy*-1-*thio*-α-*lincosaminide* (XXXI), elongated platelets from acetonitrile, m.p. 194—196°, $[\alpha]_D$ +252° (*c* 0·74 in H_2O), *m/e* 282 ($M^+ + 1$), 281 (M^+), 237 (M^+ — MeCHO), 234 (M^+ — SMe), and 208 (M^+ — MeĊHOEt) (Found: C, 46·7; H, 8·1; N, 5·3; S, 11·3. $C_{11}H_{23}NO_5S$ requires C, 46·95; H, 8·2; N, 5·0; S, 11·4%); *methyl* (7S)-7-*deoxy*-7-*isopropoxy*-1-*thio*-α-*lincosaminide* (XXXII), plates from acetonitrile, m.p. 212—213°, $[\alpha]_D$ +255° (*c* 0·38 in H_2O), *m/e* 296 ($M^+ + 1$), 295 (M^+), 280 (M^+ — Me), 248 (M^+ — SMe), and 208 (M^+ — MeĊHOPr^i) (Found: C, 48·5; H, 8·55; N, 4·8; S, 10·8. $C_{12}H_{25}NO_5S$ requires C, 48·8; H, 8·5; N, 4·7; S, 10·9%); *methyl* (7S)-7-*deoxy*-7-(2-*hydroxyethoxy*)-1-*thio*-α-*lincosaminide* (XXXIII), platelets from methanol–acetonitrile, m.p. 178·5—179·5°, $[\alpha]_D$ +243° (*c* 0·66 in H_2O), *m/e* 297 (M^+), 266 ($M^+ - CH_2OH$), 250 (M^+ — SMe), and 208 (M^+ — MeĊHOCH$_2$CH$_2$OH) (Found: C, 44·4; H, 8·0; N, 4·6; S, 10·5. $C_{11}H_{23}NO_6S$ requires C, 44·4; H, 7·8; N, 4·7; S, 10·8%); and *methyl* (7S)-7-*deoxy*-7-(2-*methoxyethoxy*)-1-*thio*-α-*lincosaminide* (XXXIV), plates from acetonitrile, m.p. 178—179°, $[\alpha]_D$ +231° (*c* 0·83 in H_2O), *m/e* 296 (M^+ — Me), 280 (M^+ — OMe), 264 (M^+ — SMe), and 208 (M^+ — MeĊHOCH$_2$CH$_2$OMe) (Found: C, 46·6; H, 8·3; N, 4·8; S, 10·6. $C_{12}H_{25}NO_6S$ requires C, 46·3; H, 8·1; N, 4·5; S, 10·3%).

Methyl (7S)-7-*Deoxy*-7-[$^2H_3$]*methoxy*-1-*thio*-α-*lincosaminide* (XXXVI).—Replacement of methanol by [$^2H_4$]methanol (99% isotopically pure; 10 g) in the reaction with the tetra-acetylepimine (XXI) (2 g) in the presence of acetic acid gave the crystalline tetra-acetyl-[$^2H_3$]methoxy-analogue (1·71 g), needles (from ethyl acetate–Skellysolve B), m.p. 243—244°, $[\alpha]_D$ +197° (*c* 0·91 in $CHCl_3$), $\nu_{max.}$ 2220, 2180, 2100, and 2050 (C–D), 1750 (ester), 1650 (amide I), and 1570 cm^{-1} (amide II), n.m.r. spectrum superimposable on that of the methoxy-tetra-acetate (VIII) except for the absence of the MeO peak at δ ($CHCl_3$) 3·30.

Hydrazinolysis of the [$^2H_3$]methoxytetra-acetate gave the amino-[$^2H_3$]methoxy-sugar (XXXVI), needles (from acetonitrile), m.p. 149·5—150°; n.m.r. spectrum superimposable on that of the free amino-methoxy-sugar (XXIX) except for the absence of the MeO peak at δ (D_2O) 3·42.

(7S)-7-*Deoxy*-7-*methoxylincomycin* (*Methyl* 6,7,8-*Trideoxy*-7-*methoxy*-6-(1-*methyl*-trans-4-*propyl*-L-*pyrrolidin*-2-*ylcarbonylamino*)-1-*thio*-L-threo-α-D-galacto-*octopyranoside*) (XXXVII).—Triethylamine (2·89 g, 28·34 mmol) was added to a stirred suspension of 1-methyl-*trans*-4-propyl-L-proline [7] (2·70 g, 12·88 mmol) in acetonitrile (90 ml) under anhydrous conditions. The solution was cooled to −5° in ice–methanol, and isobutyl chloroformate (1·78 g, 12·88 mmol) was added slowly. After 20 min, a solution of the methoxy-sugar (XXIX) (1·74 g, 6·44 mmol) in water (10 ml) was added rapidly, and stirring was continued for 1 h without further cooling. T.l.c. (methanol–chloroform, 1 : 7) showed the absence of methoxy-sugar (R_F 0·09) and the presence of a major product, R_F 0·68. Chromatography in methanol–chloroform (1 : 15) gave the free base as a syrup, which was dissolved in water; the pH was adjusted to 4·5 with hydrochloric acid (N) and the solution was lyophilised to give the amorphous *hydrochloride* of (XXXVII), $[\alpha]_D$ +117° (*c* 0·96 in H_2O), $\nu_{max.}$ 1675 (amide I) and 1550 cm^{-1} (amide II), *m/e*



(*a*) (*b*)

420 (M^+ of free base), 405 (M^+ — Me), 373 (M^+ — SMe), 271 [ion (*a*)], and 126 [ion (*b*)] [Found (corr. for 5·14% water): C, 49·9; H, 8·0; Cl, 7·5; N, 6·2; S, 6·7. $C_{19}H_{36}N_2O_6S$,HCl requires C, 49·9; H, 8·2; Cl, 7·8; N, 6·1; S, 7·0%].

By the foregoing procedure, the following analogues were obtained from the corresponding amino-sugars.

(7*S*)-7-*Deoxy*-7-*ethoxylincomycin* (XXXVIII).—The free base (960 mg, syrup; R_F 0·26) was obtained by chromatography (methanol–chloroform, 1 : 20) from the ethoxy-amine (1·53 g), and was converted into the amorphous *hydrochloride* of (XXXVIII), $[\alpha]_D$ +109° (*c* 0·98 in H_2O), $\nu_{max.}$ 1675 (amide I) and 1550 cm^{-1} (amide II), *m/e* 434 (M^+ of free base), 419 (M^+ — Me), 387 (M^+ — SMe), 361 (M^+ — MeĊHOEt), and 126 [ion (*b*)] [Found (corr. for 5·07% water): C, 50·9; H, 8·2; Cl, 7·6; N, 5·6; S, 6·95. $C_{20}H_{38}N_2O_6S$,HCl requires C, 51·0; H, 8·35; Cl, 7·5; N, 5·95; S, 6·8%].

(7S)-7-*Deoxy*-7-*isopropoxylincomycin* (XXXIX).—The

isopropoxy-amine (2·92 g) gave the free base (syrup, 1·40 g; R_F 0·22) after chromatography in ethyl acetate, and was converted into the amorphous *hydrochloride* of (XXXIX), $[\alpha]_D$ +85° (*c* 0·90 in H_2O), $\nu_{max.}$ 1660 (amide I) and 1570 cm^{-1} (amide II), *m/e* 448 (M^+ of free base), 401 (M^+ — SMe), 299 [isopropoxy-analogue of ion (*a*)], and 126 [ion (*b*)] [Found (corr. for 4·36% water): C, 51·7; H, 8·3; Cl, 7·3; N, 5·6; S, 6·35. $C_{21}H_{40}N_2O_6S$,HCl requires C, 52·0; H, 8·5; Cl, 7·3; N, 5·8; S, 6·6%].

(7S)-7-*Deoxy*-7-(2-*hydroxyethoxy*)*lincomycin* (XL).—The free base (syrup, 1·28 g; R_F 0·28) was obtained from the corresponding amino-sugar (1·40 g) by chromatography in methanol–chloroform (1 : 10), and was converted into the amorphous *hydrochloride* of (XL), $[\alpha]_D$ +107° (*c* 1·10 in H_2O), $\nu_{max.}$ 1675 and 1550 cm^{-1} (amide I and II), *m/e* 450 (M^+ of free base), 419 (M^+ — $\dot{C}H_2OH$), 403 (M^+ — SMe), 301 [2-hydroxyethoxy-analogue of ion (*a*)], and 126 [ion (*b*)] [Found (corr. for 2·11% water): C, 49·6; H, 7·85; Cl, 7·8; N, 5·5; S, 6·5. $C_{20}H_{38}N_2O_7S$,HCl requires C, 49·3; H, 8·1; Cl, 7·3; N, 5·75; S, 6·6%].

(7S)-7-*Deoxy*-7-(2-*methoxyethoxy*)*lincomycin* (XLI).—The free base (syrup, 480 mg; R_F 0·23) was obtained from the methoxyethoxy-amine (660 mg) following chromatography in ethyl acetate–methanol (100 : 5) and was converted into the amorphous *hydrochloride* of (XLI), $[\alpha]_D$ +91° (*c* 0·57 in H_2O), $\nu_{max.}$ 1660 and 1540 cm^{-1} (amide I and II), *m/e* 464 (M^+ of free base), 417 (M^+ — SMe), 315 [2-methoxyethoxy-analogue of ion (*a*)], and 126 [ion (*b*)] [Found (corr. for 4·17% water): C, 50·5; H, 8·6; Cl, 7·5; N, 5·3; S, 5·9. $C_{21}H_{40}N_2O_7S$,HCl requires C, 50·3; H, 8·25; Cl, 7·1; N, 5·6; S, 6·4%].

I acknowledge gratefully the analytical work of Dr. A. A. Forist and his associates, the mass spectral results of Drs. M. Grostic and L. Baczynskyj, the n.m.r. results of Dr. G. Slomp and Mr. S. A. Mizsak, the i.r. results of Mr. P. A. Meulman, the antibacterial assays of Dr. W. T. Sokolski and Mr. C. Lewis, the encouragment of Dr. T. E. Eble, and the technical assistance of Mr. N. E. Barry.

[3/1867 *Received*, 10*th September*, 1973]