Modifications of Lincomycin involving the Carbohydrate Portion. Part II.[1] Analogues with D-*gluco*- and D-*ido*-Stereochemistry

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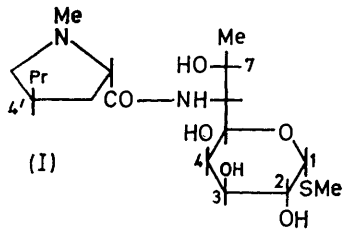
Preferential *O*-butyroylation of the antibiotic lincomycin followed by *O*-methylsulphonylation led to the 2,3,7-tri-*O*-butyrate 4-*O*-methanesulphonate. Reaction with sodium benzoate in *NN*-dimethylformamide occurred with inversion of configuration at C-4; transesterification gave the *gluco*-analogue of the antibiotic. Protection of the hydroxy-groups of lincomycin at C-3 and C-4 by acetonide formation and at C-7 by *O*-triphenylmethylation, followed by *O*-methylsulphonylation and mild acidic hydrolysis, gave lincomycin 2-*O*-methanesulphonate. This sulphonate reacted with basic nucleophiles, *via* the *talo*-epoxide, to yield exclusively analogues of *ido*-stereochemistry. The influence of such stereochemical changes on antibacterial activity in this series is discussed.

LINCOMYCIN,† an antibiotic produced by *Streptomyces lincolnensis*,[2] is methyl 6,8-dideoxy-6-(1-methyl-*trans*-4-propyl-L-pyrrolidin-2-ylcarbonylamino)-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (I).[3]



(I)

While replacement of the methylthio-group at C-1 by ethylthio-[4] results in an analogue at least equivalent in activity to lincomycin, the markedly decreased activity shown by the 2-deoxy-analogue,[1] and the virtual absence of activity in *in vitro* tests of the 2-*O*-methyl ether,[1] the 1-demethylthio-1-hydroxy-,[1,5] the *S*-oxide,[5] and the β-anomeric[1] analogues, indicated highly specific group and stereochemical requirements about C-1. In contrast, changes at the 4'-[6] and 7-[7] positions can result in materially increased activity and broadening of the antibacterial spectrum. It was of interest, therefore, to determine the effect of altered configuration and substituents in the D-*galacto*-portion of lincomycin on the antibacterial activity. For this purpose, the D-*gluco*- and D-*ido*-configurations were chosen, involving stereochemical inversions at positions 4, and 2 simultaneously with 3, respectively.

Of the four secondary hydroxy-groups in the lincomycin molecule, that at position 7 would not be expected to be subject to steric hindrance; in the most stable ($^4C_1$) conformation of the ring, those at positions 2 and 3 are equatorial, while that at position 4 is axial. The less rapid acylation of axial compared to equatorial hydroxy-groups has been established,[8] so that selective acylation of lincomycin to give a 2,3,7-tri-*O*-acylate could be envisaged, and prior preliminary results[9] had indicated the feasibility of such selective acylation. Furthermore, Williams and Richardson[10] have shown that methyl α-D-galactopyranoside can be tri-*O*-benzoylated to give the 2,3,6-triester, and that the order of reactivity of the secondary hydroxy-groups is 2-OH, 3-OH > 4-OH. (That factors other than the configuration of the hydroxy-groups can assume importance in selective acylations of pyranosides, is also stressed by these authors[10] in view of their demonstration of the formation of 2,3,6-tri-*O*-benzoates under the same conditions from methyl-α-D-*manno*- and *gluco*-pyranosides.)

While the acylation of lincomycin in pyridine with acetic anhydride at room temperature gave no evidence of selective acetylation, as indicated by t.l.c., acylation with 10 equiv. of butyric anhydride gave two major products, with R_F values consonant with tetra- and tri-*O*-butyroyl derivatives, readily separated by countercurrent distribution. The more polar product, isolated crystalline in 57% yield, was shown by elemental analysis to be a tributyrate: peaks in the mass spectrum at *m/e* 616 and 327 correspond to the molecular ion and the ion (II), demonstrating that the 7-hydroxy-group had undergone acylation, and giving the probable structure (III) for the tri-*O*-butyrate.

Reaction of the tributyrate (III) in pyridine at room temperature with 4 equiv. of methanesulphonyl chloride gave, after 60 h, a syrupy product not distinguished from starting material by t.l.c., but showing both hydroxy and sulphonate absorption ($\nu_{max.}$ 1360 cm^{-1}), indicating that sulphonylation had occurred to some extent. Countercurrent distribution resulted in the separation of starting material (11%) from the methanesulphonate tributyrate (IV), isolated in 87% yield. Mass spectral data supported this structure, showing peaks at *m/e*

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

[1] Part I, B. Bannister, preceding paper.

[2] D. J. Mason, A. Dietz, and C. DeBoer, *Antimicrobial Agents and Chemotherapy*, 1962, 554.

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

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

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

[6] B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *J. Medicin. Chem.*, 1967, **10**, 355.

[7] R. D. Birkenmeyer and F. Kagan, *J. Medicin. Chem.*, 1970, **13**, 616.

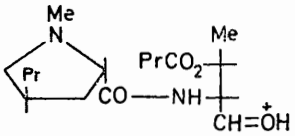
[8] E. L. Eliel and C. A. Lukach, *J. Amer. Chem. Soc.*, 1957, **79**, 5986.

[9] Dr. W. Morozowich, The Upjohn Company, personal communication; W. E. Hamlin, *J. Pharm. Sci.*, 1969, **58**, 1291.

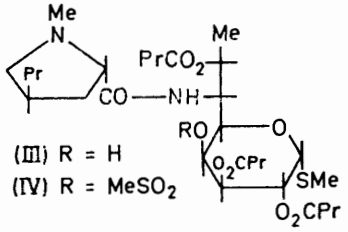
[10] (*a*) J. M. Williams and A. C. Richardson, *Tetrahedron*, 1967, **23**, 1369; (*b*) A. C. Richardson and J. M. Williams, *ibid.*, p. 1641.

694 (M^+), 579 [the ion (V)], and 327 [the ion (II)]. The n.m.r. spectrum was too complex readily to be analysed.

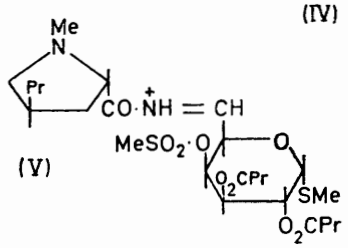
Displacement of the methylsulphonyloxy-group by sodium benzoate in boiling *NN*-dimethylformamide [11] gave the inverted benzoate (VI) in 65% yield, which was converted into the desired lincomycin analogue (VII) of D-*gluco*-configuration, by Zemplen de-esterification; [12] the product was isolated as its amorphous hydrochloride. The assignment of the 4-*epi*-structure to this product (VII) is based on the evidence presented concerning the position of the free hydroxy-group in the tributyrate (III), and the obviously hindered nature of this hydroxy-group toward methylsulphonylation, which would not be expected were it at C-2 or C-3. Further, intermolecular displacements of pyranoside 2-sulphonates are unknown, owing to the electron-withdrawing effect of the anomeric centre,[13] together with that of the ester group at C-3,[14] although an example of the displacement of a 2-sulphonate in a 3-deoxypyranoside by sodium azide has been reported.[15] Were the methylsulphonyloxy-group at C-3, the axial methylthio-group at C-1 in the methanesulphonate tributyrate would be expected to inhibit the rearward attack of the benzoate



(II)

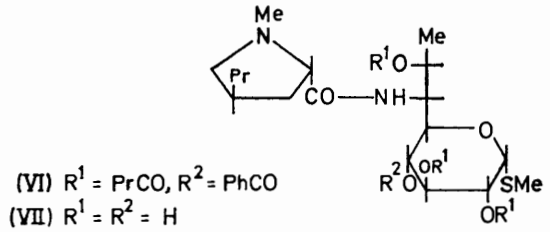


(III) R = H
(IV) R = $MeSO_2$



(V)

anion by a 1,3-diaxial interaction between the approaching nucleophile and the ring substituent.[13,16] Therefore, the ready displacement of the leaving group is further indication that the group is at C-4. This displacement of the 4-sulphonate is not expected to be complicated by ring contraction to a furanoside, since this has been observed as a significant reaction only in the case of 4-*O*-sulphonylmannopyranoside derivatives,[17] owing to the overcrowding imposed by the axial 2-substituent.



(VI) R^1 = PrCO, R^2 = PhCO
(VII) R^1 = R^2 = H

The intermediate desired for analogues involving D-*ido*-stereochemistry was a lincomycin 2-*O*-sulphonate, since this would be expected to give the D-*talo*-2,3-epoxide on treatment with base. Morozowich and his co-workers [18] have described 3,4-*O*-anisylidene-7-*O*-(triphenylmethyl)lincomycin (VIII), in which only the hydroxy-group at C-2 is unprotected. Reaction in pyridine with methanesulphonyl chloride gave the fully protected 2-*O*-methanesulphonate (IX), which was hydrolysed to the crystalline lincomycin 2-*O*-methanesulphonate (X) in hot aqueous acetic acid.

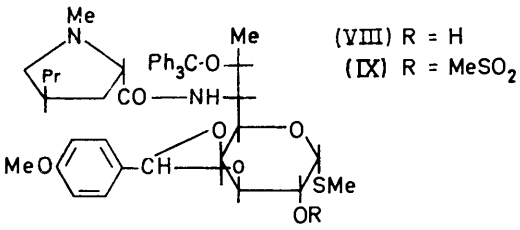
From earlier work,[3b] 3,4-*O*-isopropylidenelincomycin (XI) was available, making it more convenient to operate in this than in the anisylidene series. Reaction in boiling acetone with an excess of triphenylmethyl bromide in the presence of triethylamine gave the 7-*O*-triphenylmethyl derivative (XII); conversion into the methanesulphonate (XIII) and hydrolysis gave a product identical to that (X) from the anisylidene route. Whereas hydrolysis of the 2-hydroxy-compound (XII) to lincomycin occurred rapidly in aqueous hydrochloric acid, hydrolysis of the sulphonate derivative (XIII) resulted in the rapid cleavage of the triphenylmethyl ether to give (XIV), but the removal of the isopropylidene group was retarded greatly by the presence of the 2-sulphonyloxy-substituent, although the yield of the desired product (X) was high.

In the related *O*-alkylation of methyl *N*-acetyl-3,4-*O*-isopropylidene-1-thiolincosaminide (XV) with methyl iodide and base, selective *O*-methylation occurs at the 2-position to give (XVI),[1] in keeping with the observed enhancement of reactivity of the 2-hydroxy-group in carbohydrates, a consequence of some form of activation [10a] by the anomeric centre. That *O*-triphenylmethylation occurs exclusively at the 7-position indicates that this effect can be negated by steric factors.

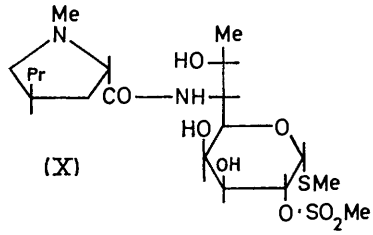
Treatment of the 2-*O*-methanesulphonate (X) with methanolic sodium methoxide under reflux gave one product only (t.l.c.), isolated as a syrup, and shown to contain

[11] E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.* 1959, **24**, 1618.
[12] G. Zemplen and E. Pacsu, *Ber.*, 1929, **62**, 1613.
[13] L. Hough and A. C. Richardson, in ' Rodd's Chemistry of Carbon Compounds,' ed. S. Coffey, 2nd edn., Elsevier, New York, 1967, vol. 1F, ch. 23, pp. 396—407.
[14] D. H. Ball and F. W. Parish, *Adv. Carbohydrate Chem.*, 1969, **24**, 139.
[15] M. Nakajima, H. Shibata, K. Kitahara, S. Takahashi, and A. Hasegawa, *Tetrahedron Letters*, 1968, 2271.
[16] N. A. Hughes and P. R. H. Speakman, *J. Chem. Soc.*, 1965, 2236.
[17] C. L. Stevens, R. P. Glinski, K. G. Taylor, P. Blumbergs, and F. Sirokman, *J. Amer. Chem. Soc.*, 1966, **88**, 2073; C. L. Stevens, R. P. Glinski, G. W. Gutowski, and J. P. Dickerson, *Tetrahedron Letters*, 1967, 649.
[18] W. Morozowich, D. J. Lamb, H. A. Karnes, F. A. MacKellar, C. Lewis, K. F. Stern, and E. L. Rowe, *J. Pharm. Sci.*, 1969, **58**, 1485.

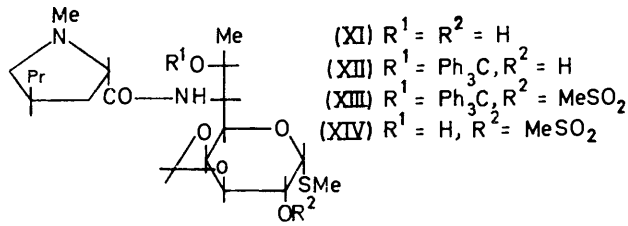
a methoxy-group by n.m.r. At room temperature, the reaction gave two products, separated by chromatography, the more polar not being distinguished from the



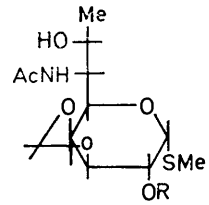
(VIII) R = H
(IX) R = $MeSO_2$



(X)



(XI) $R^1 = R^2 = H$
(XII) $R^1 = Ph_3C, R^2 = H$
(XIII) $R^1 = Ph_3C, R^2 = MeSO_2$
(XIV) $R^1 = H, R^2 = MeSO_2$



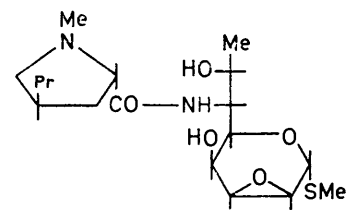
(XV) R = H
(XVI) R = Me

sole product formed under reflux. The less polar product was converted into the more polar on heating in methoxide–methanol, and therefore must be the 2,3-epoxide (XVII). Exclusive diequatorial opening of the epoxide is unlikely in view of the common applicability of the Fürst–Plattner rule[19] to the ring-opening of carbohydrate epoxides;[20] furthermore, had it occurred, it would have resulted in 2-*O*-methyl-lincomycin,[1] from which the product was distinguished readily by t.l.c. As mentioned with reference to the D-*gluco*-analogue, simple S_N2 displacement of the 2-*O*-sulphonate is inherently improbable,[13] and was eliminated by the fact that the same product resulted from the opening of the intermediate 2,3-epoxide, and the failure of this product to form an acetonide, indicating the lack of contiguous *cis*-hydroxy-groups. The product must, therefore, result from the exclusive 1,2-diaxial opening of the *talo*-epoxide, and be the 3-*O*-methyl-D-*ido*-analogue (XVIII). While the predominance of the diaxial opening of an epoxide is common, exclusive opening in this manner is unusual. It was, however, noted by Wiggins[21] in the methoxide-opening of the *talo*-2,3-epoxide derived from methyl 4,6-*O*-benzylidene-α-D-galactopyranoside 2-*O*-toluene-*p*-sulphonate, whereas the epoxide of the β-anomer gave both the idoside and the galactoside.

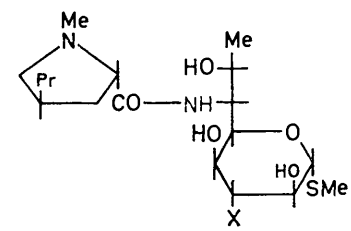
Reaction of the methanesulphonate (X) with aqueous sodium hydroxide at room temperature gave the oxide (XVII); at elevated temperatures, the D-*ido*-derivative (XIX) only was obtained. Analogous reactions in methanol with sodium methanethiolate, ammonia, and dimethylamine yielded the 3-deoxy-3-(methylthio)- (XX), the 3-amino-3-deoxy- (XXI), and the 3-deoxy-3-(dimethylamino)-D-*ido*- (XXII) derivatives, respectively. With sodium azide in boiling *NN*-dimethylformamide, the 3-azido-3-deoxy-D-*ido*-derivative (XXIII) was formed, and gave, on catalytic reduction, the amine (XXI). In no case was there any indication of the formation of other products.

All the free bases were syrups; the 3-methoxy-compound (XVIII) was isolated as its crystalline hydrochloride and the others were obtained as amorphous hydrochlorides by lyophilisation from aqueous solution. Attempted conversion of the 2,3-anhydro-*talo*-derivative into its hydrochloride was accompanied by ring opening, indicated by the presence of two anomeric proton peaks in the n.m.r. spectrum of the lyophilized salt.

Further confirmation of the D-*ido*-structure of these products was obtained from studies of periodate oxidation. With lincomycin hydrochloride, this determination is difficult to quantify because of fading end-points[22] but, in aqueous solution, 4 mol. equiv. were



(XVII)



(XVIII) X = OMe
(XIX) X = OH
(XX) X = SMe
(XXI) X = NH_2
(XXII) X = NMe_2
(XXIII) X = N_3

consumed in 1 h, with a fifth over the course of 24 h. Some doubt existed of the extent of oxidation of the methylthio-group, which was investigated with 3,4-*O*-isopropylidenelincomycin and lincomycin 2,3,4,7-tetra-*O*-acetate,[22] in which only the thioglycosidic sulphur

[19] A. Fürst and P. A. Plattner, 12th Internat. Congr. Pure and Appl. Chem., New York, 1951, abstracts, p. 409.
[20] J. A. Mills, *Adv. Carbohydrate Chem.*, 1955, **10**, 51.
[21] L. F. Wiggins, *J. Chem. Soc.*, 1944, 522.
[22] R. R. Herr and G. Slomp, *J. Amer. Chem. Soc.*, 1967, **89**, 2444.

atom is expected to undergo oxidation. The titration method of Dyer [23] was used. At the end of 1 h, these compounds had consumed 1·25 and 1·0 mol. equiv. of periodate, respectively, demonstrating that, under these conditions, the methylthio-group is oxidized only to the sulphoxide. 2-*O*-Methyl-lincomycin [1] consumed 2·1 mol. equiv., as expected, while the methylthio-compound (XX) consumed 1·8 mol. equiv. and the *O*-methyl derivative (XVIII) consumed 1·0 mol. equiv., demonstrating clearly the presence of the substituent at the 3-position.

Antibacterial Activity.—All the analogues of lincomycin reported here virtually are devoid of antibacterial activity, showing minimum inhibitory concentrations >200 μg ml^{-1}. Hence, since even the single change of configuration of the 4-hydroxy-group to give the D-*gluco*-analogue destroys activity, more than the immediate environment of the 1-position is critical for retention of activity.

EXPERIMENTAL

General experimental procedures have been described previously.[1]

Lincomycin 2,3,7-*Tri*-O-*butyrate* (III).—Lincomycin hydrochloride monohydrate (34·06 g, 76·3 mmol) in pyridine (350 ml) was stirred with butyric anhydride (121 g, 0·763 mol) for 18 h at room temperature, and solvent was removed as completely as possible by distillation at 40° under high vacuum. The residue was dissolved in methylene chloride, washed with saturated aqueous sodium hydrogen carbonate and twice with water, and dried (Na_2SO_4). T.l.c. (acetone–Skellysolve B, 1 : 2) showed two major products of R_F 0·43 and 0·62. Removal of solvent left a syrup (56·3 g) which was subjected to countercurrent distribution (500 transfers) in the system water–ethanol–ethyl acetate–cyclohexane (1 : 4 : 1 : 4). Analysis showed the desired product at K* 1·07; the less polar tetrabutyrate, K 3·38, was discarded. The colourless syrupy residue (26·22 g) obtained on removal of the solvent was extremely soluble in all solvents investigated, but crystallized slowly from Skellysolve B at 0° as *needles*, m.p. 53—54°, $[\alpha]_D$ +144° (*c* 0·845 in $CHCl_3$), $\nu_{max.}$ 3240 (OH, NH), 1740 (ester C=O), 1650 (amide I), and 1520 (amide II) cm^{-1}, *m/e* 616 (M^+), 601 ($M^+ - CH_3$), 569 ($M^+ - SCH_3$), 327 [$M^+ - 289$; see formula (II)] (Found: C, 58·6; H, 8·7; N, 4·2; S, 5·2. $C_{30}H_{52}N_2O_9S$ requires C, 58·4; H, 8·5; N, 4·5; S, 5·2%).

Lincomycin 2,3,7-*Tri*-O-*butyrate* 4-O-*Methanesulphonate* (IV).—To a stirred solution of the tributyrate (III) (13·11 g, 21·3 mmol) in pyridine (200 ml) at 0°, methanesulphonyl chloride (9·5 g, 85·2 mmol) was added slowly; the mixture was stored at room temperature for 2 days. The solvent was removed and the residue was dissolved in methylene chloride and stirred with an excess of saturated aqueous sodium hydrogen carbonate. The organic extract was washed with saturated aqueous sodium chloride and dried (Na_2SO_4). Solvent removal left a syrup (14·5 g), which was not distinguished from starting material by t.l.c. (acetone–Skellysolve B, 1 : 2; R_F 0·55). This syrup was subjected to 1000 transfers in a 500-tube countercurrent distributor in the system water–ethanol–ethyl acetate–cyclohexane (1 : 4 : 1 : 4). Starting material (K 1·02; 1·46 g) was obtained from tubes 465—45, and identified by i.r. comparison; product (K 1·44; 12·8 g, 87%) was obtained from tubes 50—130, as a *syrup*, $[\alpha]_D$ +122° (*c* 1·02 in $CHCl_3$), $\nu_{max.}$ (syrup) 1740 (ester C=O), 1677 (amide I), 1508 (amide II), and 1360 ($-SO_3-$) cm^{-1}, *m/e* 694 (M^+), 679 ($M^+ - CH_3$), 647 ($M^+ - SCH_3$), 579 [$M^+ - 115$; see formula (V)], 327 [$M^+ - 367$; formula (II)] (Found: C, 53·6; H, 7·7; N, 4·2; S, 9·0. $C_{31}H_{54}N_2O_{11}S_2$ requires C, 53·6; H, 7·8; N, 4·0; S, 9·2%).

4-epi-*Lincomycin* 2,3,7-*Tri*-O-*butyrate* 4-O-*Benzoate* [*Methyl* 6,8-*Dideoxy*-6-(1-*methyl*-trans-4-*propyl*-L-*pyrrolidin*-2-*ylcarbonylamino*)-1-*thio*-D-erythro-α-D-gluco-*octopyranoside* 2,3,7-*Tri*-O-*butyrate* 4-O-*Benzoate*] (VI).—The tributyrate 4-methanesulphonate (3·75 g, 6·3 mmol) and anhydrous sodium benzoate (2·72 g, 22·1 mmol) in *NN*-dimethylformamide (110 ml) were stirred in an oil-bath at 150° for 20 h, giving a dark mixture from which solvent was removed at 90° and <1 mmHg. A solution of the residue in methylene chloride was washed with water and dried (Na_2SO_4). T.l.c. (acetone–Skellysolve B, 1 : 5) showed some starting material (R_F 0·09) and a major zone of product (R_F 0·21). Removal of solvent gave a dark syrup (4·05 g), chromatographed on silica gel in the same system to yield the benzoate as a colourless *syrup* (2·54 g, 65%), $[\alpha]_D$ +81° (*c* 0·745 in $CHCl_3$); $\nu_{max.}$ (syrup) 1740 (ester C=O), 1685 (amide I), 1605, 1585 (aromatic C=C), and 1500 (amide II) cm^{-1}; $\lambda_{max.}$ (EtOH) 231·5 (ε 12,450), 274 (1570), and 282·5sh (1310) nm, *m/e* 720 (M^+), 705 ($M^+ - CH_3$), 673 ($M^+ - SCH_3$), and 327 [see formula (II)] (Found: C, 61·8; H, 7·9; N, 4·0; S, 3·9. $C_{37}H_{56}N_2O_{10}S$ requires C, 61·6; H, 7·8; N, 3·9; S, 4·45%).

4-epi-*Lincomycin* [*Methyl* 6,8-*Dideoxy*-6-(1-*methyl*-trans-4-*propyl*-L-*pyrrolidin*-2-*ylcarbonylamino*)-1-*thio*-D-erythro-α-D-gluco-*octopyranoside*] (VII).—The tetra-ester (VI) (2·0 g) in methanol (30 ml) containing sodium methoxide (25% solution in methanol; 0·3 ml) was kept overnight at room temperature; t.l.c. (methanol–chloroform, 1 : 7) showed the absence of starting material (R_F 0·91) and a major new zone (R_F 0·12). The solution was neutralised with solid carbon dioxide and the solvent was removed, giving a syrup smelling of methyl benzoate. Trituration with ether gave a solid (1·14 g), which was chromatographed (silica gel) in methanol–chloroform (1 : 5). The desire product (R_F 0·24; 1·01 g, 70%) was dissolved in water, the pH was adjusted to 4·5 with hydrochloric acid (N), and the solution was lyophilised, giving an amorphous *hydrochloride*, $[\alpha]_D$ +123° (*c* 0·97 in H_2O), $\nu_{max.}$ (mull) 1670 (amide I) and 1560 (amide II) cm^{-1}, *m/e* 406 (M^+, free base), 391 ($M^+ - CH_3$), 388 ($M^+ - H_2O$), and 359 ($M^+ - SCH_3$) [Found (corrected for 2·72% water): C, 48·5; H, 8·2; Cl, 8·3; N, 6·1; S, 7·0. $C_{18}H_{34}N_2O_6S$,HCl requires C, 48·8; H, 8·0; Cl, 8·0; N, 6·3; S, 7·2%].

3,4-O-*Anisylidene*-7-O-(*triphenylmethyl*)*lincomycin* 2-O-*Methanesulphonate* (IX).—To a stirred solution of the triphenylmethyl anisylidene derivative [18],† (VIII) (29·2 g, 38 mmol) in pyridine (450 ml) was added methanesulphonyl chloride (21·8 g, 0·146 mol) during 20 min; next day the solvent was removed to give a red-brown syrup, which was dissolved in methylene chloride and stirred with aqueous sodium hydroxide (N). The organic layer was washed with water and dried (Na_2SO_4). T.l.c. (acetone–Skellysolve B,

* See ref. 1 for a definition of K.

† Supplied by Dr. W. Morozowich of The Upjohn Company.

[23] J. R. Dyer, in 'Methods of Biochemical Analysis,' ed. D. Glick, Interscience, New York, 1956, vol. 3, p. 111.

1 : 1) showed the absence of starting material (R_F 0·59) and a new zone (R_F 0·72). Solvent was removed to give a dark syrup which was dissolved in methanol; the solution was treated with animal charcoal, filtered, and concentrated at the b.p. The product separated as colourless *needles* (23·0 g, 71·5%), m.p. 100·5—102°, $[\alpha]_D$ +80° (*c* 0·96 in $CHCl_3$), $\nu_{max.}$ 1675 (amide I), 1610, 1585, 1510 (amide II, aromatic C=C), and 1360 ($-SO_3-$) cm^{-1}; $\lambda_{max.}$ (EtOH) 223 (ε 24,800), 254sh (1200), 260sh (1500), 264sh (1600), 266 (1650), 269 (1650), and 278 (1200) nm (Found: C, 65·1; H, 6·7; N, 3·1; S, 7·6. $C_{46}H_{56}N_2O_9S_2$ requires C, 65·4; H, 6·7; N, 3·3; S, 7·6%).

3,4-O-*Isopropylidene*-7-O-(*triphenylmethyl*)*lincomycin* (XII).—3,4-*O*-Isopropylidenelincomycin [3b] (5·0 g, 10·2 mmol) was dissolved in acetone (50 ml), triethylamine (9·05 g, 81·6 mmol) and triphenylmethyl bromide (18·1 g, 51 mmol) were added, and the mixture was heated under reflux for 6 h. T.l.c. (acetone–Skellysolve B, 1 : 1) showed very little acetonide (R_F 0·28), a major zone (R_F 0·57), and the excess of reagent (R_F 0·70). Precipitated triethylammonium bromide was filtered off, and solvent was removed from the filtrate and washings to give a dark syrup; the product crystallized from acetonitrile in colourless *needles* (5·4 g, 70%). Recrystallization gave a sample of m.p. 154—155°, $[\alpha]_D$ +59° (*c* 0·74 in $CHCl_3$), $\nu_{max.}$ 1655 (amide I) and 1520 (amide II) cm^{-1}, $\lambda_{max.}$ (EtOH) 253 (ε 737), 259 (778), 262sh (703), 265sh (613), and 269sh (455) nm (Found: C, 69·9; H, 7·8; N, 4·0; S, 4·6. $C_{40}H_{52}N_2O_6S$ requires C, 69·7; H, 7·6; N, 4·1; S, 4·65%).

3,4-O-*Isopropylidene*-7-O-(*triphenylmethyl*)*lincomycin* 2-O-*Methanesulphonate* (XIII).—The triphenylmethyl isopropylidene compound (XII) (5·0 g) was converted into the methanesulphonate as described for the anisylidene analogue. The crude product was a tan solid (6·68 g), which separated from ethyl acetate in *platelets* (4·2 g, 79%), m.p. 193—195°, $[\alpha]_D$ +71° (*c* 0·74 in $CHCl_3$), $\nu_{max.}$ 1670 (amide I), 1515 (amide II), 1360, 1335, and 1180 ($-SO_3-$) cm^{-1}, $\lambda_{max.}$ (EtOH) 253 (ε 690), 259 (752), 262sh (683), 266sh (575), and 269sh (437) nm (Found: C, 64·2; H, 7·1; N, 3·6; S, 8·4. $C_{41}H_{54}N_2O_8S_2$ requires C, 64·2; H, 7·1; N, 3·65; S, 8·4%).

Lincomycin 2-O-*Methanesulphonate* (X).—(a) *From the anisylidene derivative* (IX). A solution of the protected methanesulphonate (18·0 g) in glacial acetic acid (200 ml) was diluted with water (50 ml) and heated in an oil-bath at 110° for 30 min. T.l.c. (methanol–chloroform, 1 : 15) showed a new polar zone (R_F 0·45). The solution was cooled and diluted with water (250 ml), and the precipitated triphenylmethanol was removed and washed with 50% aqueous acetic acid; solvent was removed from the filtrate. The residue was dissolved in methylene chloride and extracted with aqueous hydrochloric acid (N), the extracts were cooled to 0°, adjusted to pH 9 (50% aqueous sodium hydroxide), and extracted with methylene chloride. The latter extracts were washed with water and dried (Na_2SO_4). Removal of solvent gave a pale yellow amorphous solid (10·0 g) which separated from acetone–Skellysolve B in *needles*, m.p. 185—187° (8·2 g, 79·5%), $[\alpha]_D$ +53° (*c* 0·68 in MeOH), $\nu_{max.}$ 1635 (amide I), 1525 (amide II), 1360, and 1180 ($-SO_3-$) cm^{-1} (Found: C, 47·2; H, 7·7; N, 5·65; S, 13·2. $C_{19}H_{36}N_2O_8S_2$ requires C, 47·1; H, 7·5; N, 5·8; S, 13·2%).

(b) *From the isopropylidene derivative* (XIII). Aqueous hydrochloric acid (N; 10 ml) was added to a suspension of the protected methanesulphonate (4·3 g) in methanol (40 ml); the solid dissolved rapidly, to be replaced by a precipitate of triphenylmethanol. After 4 h t.l.c. (methanol–chloroform, 1 : 7) showed the absence of starting material (R_F 0·87, chars with sulphuric acid at 100°) and the presence of triphenylmethanol (R_F 0·87, chars only slightly), a major zone (R_F 0·79) of the acetonide methanesulphonate (XIV), and a trace of desired free methanesulphonate (R_F 0·53). After 20 h, the intermediate R_F zone had disappeared. The solution was adjusted to pH 9 (triethylamine), solvent was removed, and the residual syrup was purified as under (a) to give the product (2·15 g, 81%), m.p. 185—187°, identical with that obtained earlier.

2-epi,3-epi-3-O-*Methyl-lincomycin* [*Methyl* 6,8-*Dideoxy*-6-(1-*methyl*-trans-4-*propyl*-L-*pyrrolidin*-2-*ylcarbonylamino*)-3-O-*methyl*-1-*thio*-D-erythro-α-D-ido-*octopyranoside*](XVIII).—A solution of the 2-*O*-methanesulphonate (X) (1·59 g, 3·29 mmol) in methanol (42 ml) containing sodium methoxide [from sodium (340 mg, 14·8 mg atom)] was heated under reflux for 3 h. T.l.c. (methanol–chloroform, 1 : 7) showed the disappearance of starting material (R_F 0·50) and the formation of a new zone (R_F 0·63). Neutralization with solid carbon dioxide and removal of solvent gave a colourless syrup which was dissolved in methylene chloride; the solution was filtered from inorganic solid and solvent was removed to give a syrup (1·57 g). Chromatography (silica gel; methanol–chloroform, 1 : 15) gave pure material (1·14 g) which failed to yield a solid hydrochloride from acetone; lyophilisation of an aqueous solution adjusted to pH 4·5 with hydrochloric acid (N) gave an amorphous *solid*, $[\alpha]_D$ +158° (*c* 0·71 in H_2O), *m/e* 420 (M^+ of free base), 405 ($M^+ - CH_3$), and 373 ($M^+ - SCH_3$) [Found (corrected for 2·11% water): C, 49·7; H, 8·4; Cl, 7·8; N, 6·1; S, 7·0; OMe, 6·8. $C_{19}H_{36}N_2O_6S$,HCl requires C, 49·9; H, 8·2; Cl, 7·8; N, 6·1; S, 7·0; OMe, 6·2%].

Under the same reaction conditions except at room temperature, the 3-*O*-methyl product was accompanied by the 2,3-epoxide (XVII), described later. This epoxide in boiling methanolic sodium methoxide gave the same 3-*O*-methyl compound, identified by mass, n.m.r., and i.r. spectral comparisons.

2-epi,3-epi-*Lincomycin* (XIX) *and the* 2,3-*Anhydro*-talo-*analogue* [*Methyl* 2,3-*Anhydro*-6,8-*dideoxy*-6-(1-*methyl*-trans-4-*propyl*-L-*pyrrolidin*-2-*ylcarbonylamino*)-1-*thio*-D-erythro-α-D-talo-*octopyranoside* (XVII)].—To a stirred solution of the 2-*O*-methanesulphonate (X) (1·0 g, 2·03 mmol) in water (30 ml) at room temperature was added sodium hydroxide (420 mg, 14·8 mmol). After 20 h, t.l.c. (methanol–chloroform, 1 : 7) indicated a new zone, R_F 0·58, and the absence of methanesulphonate. Isolation as described for the 3-*O*-methyl derivative gave a colourless syrup (700 mg), showing *m/e* 388 (M^+) 343 ($M^+ - CH_3\dot{C}HOH$), and 341 ($M^+ - SCH_3$), in conformity with the 2,3-epoxide structure (XVII).

Treatment of the free base in acetone (4 ml) with hydrochloric acid (4N) (to pH 3), followed by dilution with acetone gave a colourless amorphous solid, shown to be a mixture of epoxide and material having undergone ring opening by the acid, $[\alpha]_D$ +52° (*c* 0·81 in H_2O), with two methyl doublets, two methylthio-singlets, and two anomeric hydrogen doublets in the n.m.r. spectrum; *m/e* 388, 343, and 341 as for the epoxide free base together with *m/e* 424/426 (M^+, free base of chlorohydrin), 409/411 ($M^+ - CH_3$), and 377/379 ($M^+ - SCH_3$) [Found (corrected for 2·28% water): C, 49·8; H, 7·6; Cl, 9·6; N, 6·4; S, 7·0. Calc. for $C_{18}H_{32}N_2O_5S$,HCl: C, 50·9; H, 7·8; Cl, 8·3; N,

6·6; S, 7·55. Calc. for $C_{18}H_{33}ClN_2O_5S$,HCl: C, 46·85; H, 7·4; Cl, 15·3; N, 6·1; S, 6·95%].

With the same reaction conditions but under reflux, the 2-*O*-methanesulphonate (9·72 g) yielded the D-*ido*-analogue as an amorphous *hydrochloride* (XIX) (4·76 g), $[\alpha]_D$ +81° (*c* 1·12 in H_2O), *m/e* 406 (M^+ of free base), 391 ($M^+ - CH_3$), 388 ($M^+ - H_2O$), and 359 ($M^+ - SCH_3$) [Found (corrected for 2·57% water): C, 48·7; H, 8·3; Cl, 8·05; N, 6·0; S, 7·4. $C_{18}H_{34}N_2O_6$,HCl requires C, 48·8; H, 8·0; Cl, 8·0; N, 6·3; S, 7·2%].

2-epi,3-epi-3-*Deoxy*-3-(*methylthio*)*lincomycin* (XX).—By entirely analogous procedures with methanolic sodium methanethiolate, the methanesulphonate (1·0 g) gave the analogue as its amorphous *hydrochloride* (880 mg), $[\alpha]_D$ +46° (*c* 0·70 in H_2O), *m/e* 436 (M^+ of free base), and the standard ions at $M^+ - 15$, $M^+ - 18$, and $M^+ - 47$ [Found (corrected for 4·89% water): C, 48·3; H, 8·1; Cl, 7·8; N, 5·95; S, 13·9. $C_{19}H_{35}N_2O_5S_2$,HCl requires C, 48·3; H, 7·7; Cl, 7·5; N, 5·9; S, 13·6%].

2-epi,3-epi-3-*Amino*-3-*deoxylincomycin* (XXI).—The methanesulphonate (1·0 g) in ammoniacal methanol in a sealed tube at 100° gave the product (550 mg) as an amorphous *dihydrochloride*, $[\alpha]_D$ +55° (*c* 0·54 in H_2O), *m/e* 405 (M^+ of free base) together with ions from the standard cleavages [Found (corrected for 8·49% water): C, 44·9; H, 7·9; Cl, 14·5; N, 9·0; S, 6·8. $C_{18}H_{35}N_3O_5S$,2HCl requires C, 45·2; H, 7·8; Cl, 14·8; N, 8·8; S, 6·7%].

2,epi,3-epi-3-*Deoxy*-3-*dimethylaminolincomycin* (XXII).—From the methanesulphonate (2·0 g) with methanolic dimethylamine at 100° was obtained the amorphous *dihydrochloride*, $[\alpha]_D$ +69° (*c* 1·06 in H_2O), *m/e* 433 (M^+ of free base), together with ions from the standard cleavages [Found (corrected for 6·73% water): C, 47·2; H, 8·6; Cl, 14·0; N, 8·5; S, 6·4. $C_{20}H_{39}N_3O_5S$,2HCl requires C, 47·4; H, 8·2; Cl, 14·0; N, 8·3; S, 6·3%].

2-epi,3-epi-3-*Azido*-3-*deoxylincomycin* (XXIII).—From the methanesulphonate (1·0 g) in *NN*-dimethylformamide with sodium azide (1 equiv.) at 140° for 24 h was obtained a crude product (syrup, 800 mg) showing strong absorption at 2110 cm^{-1} (N_3). Chromatography (silica gel; methanol–chloroform, 1 : 15) followed by conversion into the hydrochloride in aqueous solution and lyophilisation gave the amorphous *hydrochloride* (XXIII), $[\alpha]_D$ +86° (*c* 0·91 in H_2O) [Found (corrected for 3·68% water): C, 46·1; H, 7·65; Cl, 7·6; N, 14·8; S, 6·6. $C_{18}H_{33}N_5O_5S$,HCl requires C, 46·2; H, 7·3; Cl, 7·6; N, 15·0; S, 6·9%].

Reduction of this azide in ethanol in a Parr hydrogenator in the presence of Raney nickel gave an amino-compound identical ($[\alpha]_D$, t.l.c., and n.m.r.) with that (XXI) obtained from the methanesulphonate with ammonia.

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