Semisynthetic Macrolide Antibacterials Derived from Tylosin. Synthesis of 3-*O*-Acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin and Related Compounds, as well as the 12,13-Epoxy Derivatives

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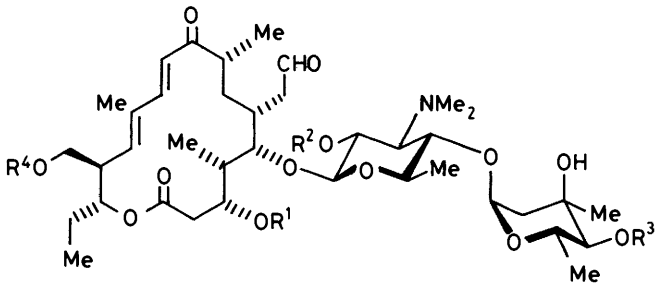
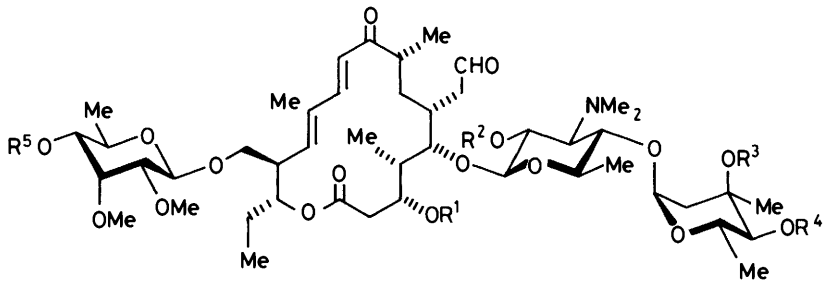
Selective acylation techniques have been developed that enable the synthesis of 3-*O*-acetyl-4″-*O*-isovaleryltylosin and 3-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin to be carried out in an efficient manner starting from tylosin. The syntheses of the 2′-*O*-acetyl, 23-*O*-acetyl, and 2′,23-di-*O*-acetyl derivatives of the latter are also described. The synthesis of key hydrazones is also described. The regio- and stereo-selective epoxidation of tylosin and its acyl derivatives afforded the 12,13-epoxy analogues, which were used to synthesize novel acylated 12,13-epoxy derivatives of 23-*O*-demycinosyltylosin.

The methodology used to synthesize 23-*O*-demycinosyltylosin (**1**) (23-DMT) in the preceding paper [1] was not compatible with the presence of 3-*O*-acyl groups in the molecule. We therefore set out to develop alternative, selective acylation strategies that could be used to synthesize certain key target derivatives of tylosin (**8**) and 23-*O*-demycinosyltylosin (**1**), namely 3-*O*-acetyl-4″-*O*-*iso*-valeryltylosin (**9**) and 3-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**2**) respectively.



(**1**) $R^1 = R^2 = R^3 = R^4 = H$
(**2**) R^1 = Ac, $R^2 = R^4 = H$, $R^3 = COCH_2CHMe_2$
(**3**) $R^1 = R^2$ = Ac, $R^3 = COCH_2CHMe_2$, $R^4 = H$
(**4**) $R^1 = R^2 = R^4$ = Ac, $R^3 = COCH_2CHMe_2$
(**5**) $R^1 = R^2$ = Ac, $R^3 = COCH_2CHMe_2$, $R^4 = COCH_2Ph$
(**6**) R^1 = Ac, R^2 = H, $R^3 = COCH_2CHMe_2$, $R^4 = COCH_2Ph$
(**7**) $R^1 = R^4$ = Ac, R^2 = H, $R^3 = COCH_2CHMe_2$

In view of the fact that vicinal 4→3 migration of the 4-acyl group in mycarose has previously been noted[2] during forcing acylation conditions, it seemed prudent to check whether such migrations would occur under the acylation conditions that we wished to use in this study. Thus methyl β-L-mycaroside (**17**) was converted into the 4-*O*-chloroacetate (**18**), which reacted with acetic anhydride and 4-dimethylaminopyridine (DMAP) in the presence of triethylamine to give only the transacylation product (**19**). The latter, on mild hydrolysis with triethylamine in methanol, afforded the 4-*O*-acetate (**20**). The latter was also prepared directly from compound (**17**) with acetic anhydride and DMAP in the presence of triethylamine, no transacylation being observed in this instance. Similar results were obtained with the α-anomer. The ^{13}C n.m.r. data are given in the Supplementary Publication. It is evident from the above results that transacylation from the 4-hydroxy group to the neighbouring 3-hydroxy group occurs only under these reaction conditions with the more reactive 4-*O*-chloroacetyl derivatives. In the case of the 4-*O*-acetyl derivatives no transacylation was observed under the same conditions. Under these mild DMAP-catalysed acylation conditions, which we needed to use to prepare the 3-*O*-acyl macrolides, we observed no acetylation at the tertiary 3-hydroxy group of the mycarose moiety either.[3] Also, in view of the differences in the reactivity of the 4‴-esters *versus* the 4″- and 3-esters observed in preliminary studies in these laboratories, there was no need to use more labile ester



(**8**) $R^1 = R^2 = R^3 = R^4 = R^5 = H$
(**9**) R^1 = Ac, $R^2 = R^3 = R^5 = H$, $R^4 = COCH_2CHMe_2$
(**10**) $R^1 = R^3 = R^4 = R^5 = H$, R^2 = Ac
(**11**) $R^1 = R^3 = R^4 = H$, $R^2 = R^5$ = Ac
(**12**) $R^1 = R^3 = H$, $R^2 = R^4 = R^5$ = Ac
(**13**) $R^1 = R^3 = H$, $R^2 = R^5$ = Ac, $R^4 = COCH_2CHMe_2$
(**14**) $R^1 = R^2 = R^5$ = Ac, $R^3 = H$, $R^4 = COCH_2CHMe_2$
(**15**) $R^1 = R^2$ = Ac, $R^3 = R^5 = H$, $R^4 = COCH_2CHMe_2$
(**16**) $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = COCH_2CHMe_2$

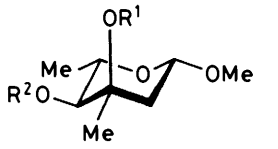
groups to protect the 4‴-hydroxy group in tylosin (**8**). We therefore proceeded to synthesize the target macrolides in the following manner.

2′-*O*-Acetyltylosin (**10**)[1] on controlled acetylation with acetic anhydride and DMAP in the presence of pyridine gave 2′,4‴-di-*O*-acetyltylosin (**11**), together with traces of 2′,4″,4‴-tri-*O*-acetyltylosin (**12**). Selective acylation of compound (**11**) with isovaleric anhydride and DMAP in the presence of triethylamine gave the desired 4″-*O*-isovalerate (**13**).

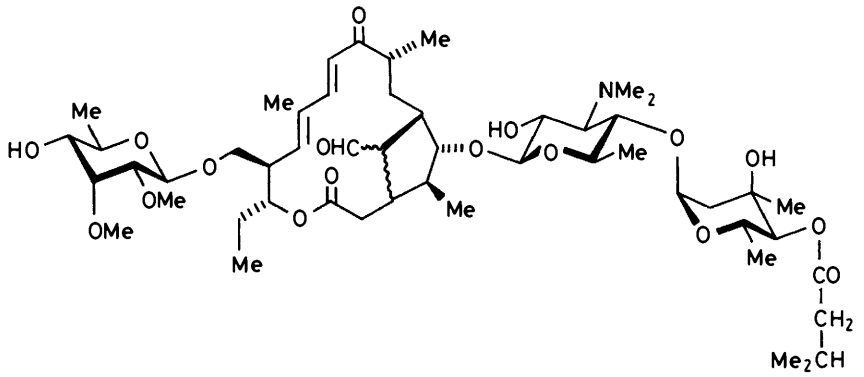
Acetylation of compound (**13**) with acetic anhydride and DMAP in the presence of triethylamine afforded the desired 2′,3,4‴-tri-*O*-acetyl-4″-*O*-isovaleryltylosin (**14**), making these the conditions of choice for preparing the 3-*O*-acetyl derivatives.[4]

Hydrolysis of compound (**14**) with 25% triethylamine in methanol at 25 °C afforded 3-deoxy-4″-*O*-isovaleryl-3,19-cyclotylosin (**21**) and the desired 3-*O*-acetyl-4″-*O*-isovaleryltylosin (**9**).[5] On reduction of the concentration of triethylamine to *ca.* 1.7%, selective deacetylation of (**14**) to give (**9**) could be achieved in high yield. Under these conditions none of the bicycloaldehyde (**21**) was formed. Base-catalysed formation of bicycloaldehydes has been noted previously.[1,6,7] Treatment of compound (**9**) with *N*-amino-4,4-dioxothiomorpholine afforded the hydrazone (**22**).

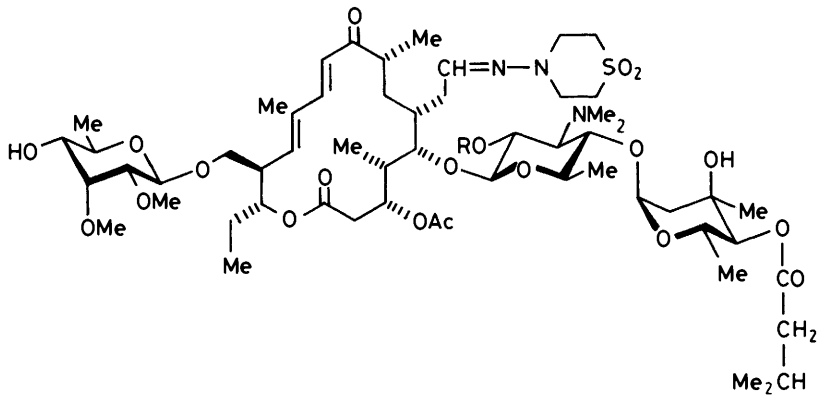
We next turned our attention to the synthesis of 3-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**2**) from compound (**9**). Selective acetylation of (**9**) gave 2′,3-di-*O*-acetyl-4″-*O*-isovaleryltylosin (**15**) which could then be subjected to Pfitzner–Moffatt oxidation conditions.[1,4,8,9] Thus compound (**15**) on oxidation using the water-soluble *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodi-imide hydrochloride at room temperature afforded the ketone (**24**) in good yield, provided the product was chromatographed rapidly. When the oxidation product was chromatographed at normal flow rates on silica gel, the enone (**25**) and 2′,3-di-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**3**) were obtained. Treatment of ketone (**24**) with triethylamine in methanol gave a mixture of products (**3**) and (**2**). Similarly, enone (**25**) on treatment with triethylamine in methanol also gave both compounds (**3**) and (**2**). If the oxidation of compound (**15**) was allowed to proceed at 25 °C for 36 h and the product was then chromatographed on silica gel at normal flow rates, a high yield of compound (**3**) was obtained. Conversion of compound (**15**) into the hydrazone (**23**), followed by oxidation and hydrolysis with triethylamine in methanol, gave both the 2′,3-di-*O*-acetyl and the 3-*O*-acetyl-DMT derivatives (**26**) and (**27**) respectively. The aldehydes (**3**) and (**2**) were also directly converted into their respective hydrazones (**26**) and (**27**) by treatment with *N*-amino-4,4-dioxothiomorpholine.



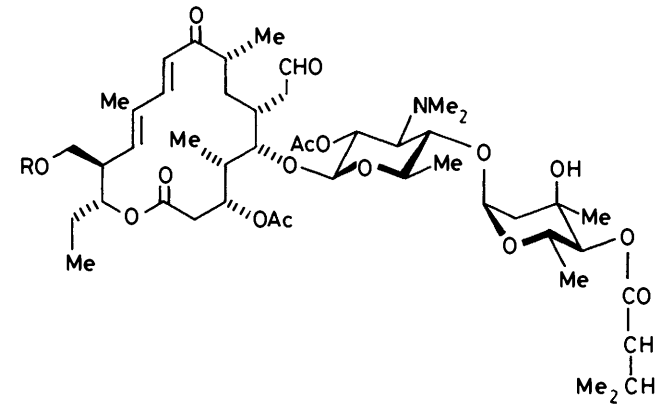
(**17**) $R^1 = R^2 = H$
(**18**) $R^1 = H$, $R^2 = COCH_2Cl$
(**19**) $R^1 = COCH_2Cl$, $R^2 = Ac$
(**20**) $R^1 = H$, $R^2 = Ac$



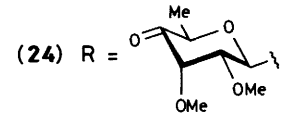
(**21**)



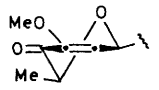
(**22**) R = H
(**23**) R = Ac

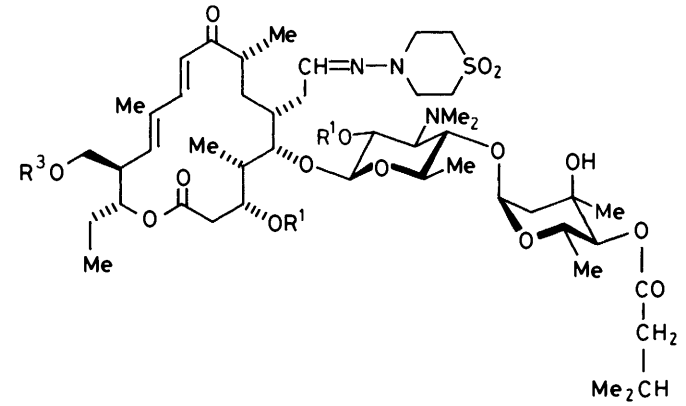


(24) R =



(25) R =



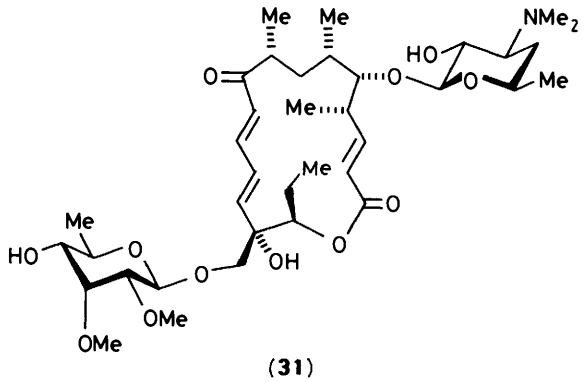


(26) $R^1 = R^2 = Ac$, $R^3 = H$
(27) $R^1 = Ac$, $R^2 = R^3 = H$
(28) $R^1 = R^2 = R^3 = Ac$
(29) $R^1 = Ac$, $R^2 = H$, $R^3 = COCH_2Ph$
(30) $R^1 = R^3 = Ac$, $R^2 = H$

Acetylation of compound (**2**) using acetic anhydride, DMAP, and pyridine afforded 2′,3,23-tri-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**4**), which was in turn converted into the hydrazone (**28**). 2′,3-Di-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**3**) on treatment with phenylacetyl chloride and pyridine gave the 23-*O*-phenylacetyl derivative (**5**). Methanolysis of the latter gave 3-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryl-23-*O*-phenylacetyltylosin (**6**), which was converted in the hydrazone (**29**). Methanolysis of compound (**3**) afforded monoacetate (**2**). When triacetate (**4**) was subjected to methanolysis, the 3,23-di-*O*-acetyl derivative (**7**) was obtained and it was converted into the hydrazone (**30**).

At the time our own work described above was nearing completion, reports appeared describing independent studies carried out at Eli Lilly and Company,[10,11] in which 23-DMT (**1**), which had been prepared by mutasynthesis,[12–14] was converted into a different series of 3,4″-acyl derivatives. The Lilly group employed a labile 23-*O*-trichloracetyl protecting group in their synthetic studies[10,11] and used different acylation conditions, for introduction of the 3- and 4″-*O*-acyl groups, to those described above. 3-*O*-Acetyl-4″-*O*-isovaleryl-23-DMT (**2**) has also recently been prepared by mutasynthesis.[15]

Having synthesized the target acyl DMT derivatives and their hydrazones, we next turned our attention to the synthesis of their 12,13-epoxy derivatives which have never been described before. During the course of mutasynthesis studies on the rosaramicins, carried out in these laboratories, it had been observed[16] that epoxidation of the 20-deoxo-12,13-de-epoxy-12,13-didehydrorosaramicin aglycone with *m*-chloroperbenzoic acid (MCPBA) led to regio- and stereo-selective epoxidation of the 12,13-double bond. The resulting epoxide was shown to have the same absolute stereochemistry as that in rosaramicin (**34**).[16,17] A subsequent U.S. patent has also claimed similar methods for preparing the 12,13-epoxide of 5-*O*-mycaminosyltylonolide.[18] At the time we started our work the epoxidation method had not been applied to dimethylamino sugar-containing macrolides in these laboratories. We therefore chose mycinamicin V (**31**)[19,20] as a model for a non-aldehydo



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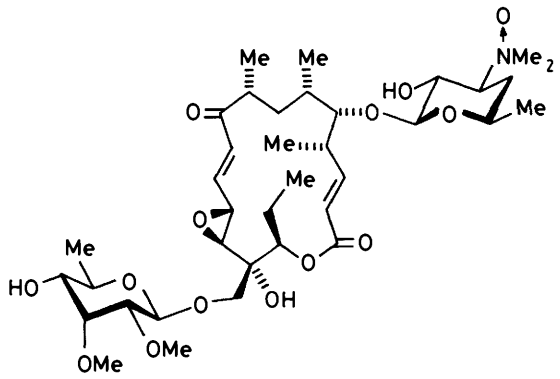

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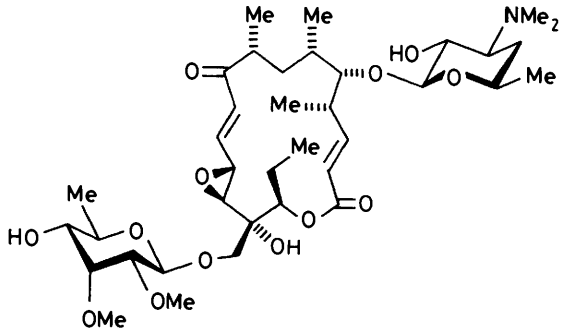


(33)

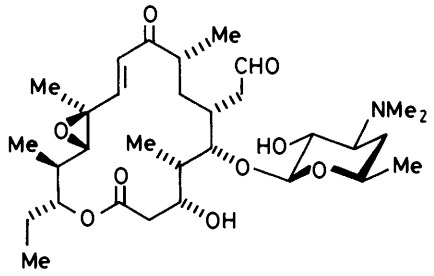
macrolide. Epoxidation of compound (**31**) with MCPBA in the presence of disodium hydrogen phosphate afforded mycinamicin II 3′-*N*-oxide (**32**). The latter was converted into the free base (**33**) on reflux with triphenylphosphine in dichloromethane as a solvent. The 12,13-epoxide was unaffected by these reaction conditions. The epoxide so obtained was identical in all respects with mycinamicin II (**33**) produced by *Micromonospora*.[19,20]

We next turned our attention to the aldehydo macrolides. Rosaramicin (**34**) on brief treatment with MCPBA gave the *N*-oxide (**35**). Reduction of rosaramicin (**34**) with titanium(III) chloride in 0.1M hydrochloric acid gave the de-epoxyrosaramicin (**37**). When rosaramicin *N*-oxide (**35**) was reduced with titanium(III) chloride in aqueous methanol at pH 1.5 a 60:40 mixture of the de-epoxyrosaramicin (**37**) and its 20-dimethyl acetal (**38**) was produced. Similarly rosaramicin (**34**) on reduction with titanium(III) chloride in aqueous methanol at pH 1 gave both compounds (**37**) and (**38**). Reduction of rosaramicin (**34**) with titanium(III) chloride in the presence of disodium hydrogen phosphate in aqueous tetrahydrofuran (THF) at pH 5.5 gave the alcohols (**39**) and (**40**). It was obvious from the above results that although titanium(III) chloride was useful as an alternative reagent for preparing de-epoxyrosaramicin (**37**)[21] from rosaramicin (**34**), it was of little use in selectively reducing the *N*-oxide group.

When rosaramicin (**34**) was allowed to react with MCPBA for longer periods of time, the 20-aldehyde underwent oxidation. The sensitive 20-aldehyde would therefore have to be protected. Thus, de-epoxyrosaramicin (**37**) was converted into the 20-dimethyl acetal (**38**) by treatment with methanol and difluoroacetic acid (DFA).[22] Epoxidation of compound (**38**), either with MCPBA alone, or with MCPBA buffered with disodium hydrogen phosphate, afforded rosaramicin 20-dimethyl acetal 3′-*N*-oxide (**36**). The latter on treatment with dil. hydrochloric acid gave rosaramicin 3′-*N*-oxide (**35**). In contrast to its reaction with titanium(III) chloride,[23,24] the latter could be cleanly converted into rosaramicin (**34**), either by heating with carbon disulphide[25] in dichloromethane, or by heating with triphenylphosphine[26] in dichloromethane. The rosaramicin (**34**) so produced was identical in all respects with the natural material produced by *Micromonospora rosea*. The epoxidation was occurring regio- and stereo-selectively at the 12,13-double bond even in the presence of disodium hydrogen phosphate as a buffer. We were now in a position to prepare the 12,13-epoxy derivatives of desmycosin (**41**), tylosin (**8**), and selected acyl derivatives of tylosin.

Brief treatment of desmycosin 20-dimethyl acetal (**42**), prepared from desmycosin (**41**),[27] with MCPBA gave the *N*-oxide (**43**). Reaction of compound (**42**) with MCPBA and disodium hydrogen phosphate at 60 °C for 23 h gave the epoxide *N*-oxide (**44**) together with small amounts of the *N*-oxide (**43**). Reaction of compound (**42**) with MCPBA alone at either 60 °C or 25 °C afforded the epoxide *N*-oxide (**44**). The latter, on being heated with triphenylphosphine in dichloromethane, gave 12,13-epoxydesmycosin 20-dimethyl acetal (**45**). Hydrolysis of compound (**45**) with dil. hydrochloric acid gave 12,13-epoxydesmycosin (**46**).

Tylosin (**8**) on treatment with methanol and DFA gave tylosin 20-dimethyl acetal (**47**), together with some desmycosin 20-dimethyl acetal (**42**) which was formed due to the lability of the mycarose moiety even under these extremely mild acidic conditions. The acetal (**47**) was treated with MCPBA buffered with disodium hydrogen phosphate to give the 12,13-epoxide *N*-oxide (**49**). The latter reacted with triphenylphosphine to give 12,13-epoxytylosin 20-dimethyl acetal (**51**). When the reaction was carried out using larger quantities of triphenylphosphine both compound (**51**) and a by-product (**58**) were formed. The FAB-MS showed an MH^+ peak at 964 mu which was 14 mu less than that of the expected product (**51**). The ^1H n.m.r. and ^{13}C n.m.r. data (Supplementary Publication) were consistent with the product (**58**) being 3′-*N*-demethyl-12,13-epoxytylosin 20-



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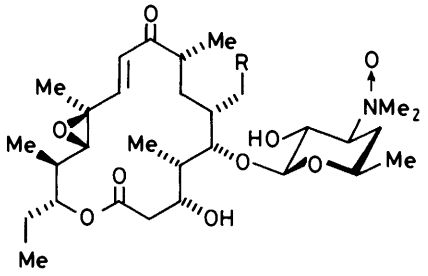
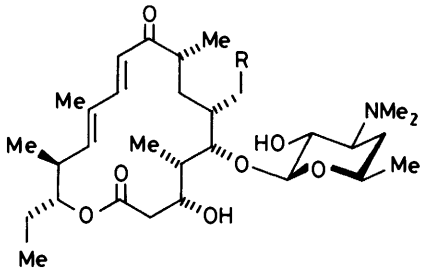
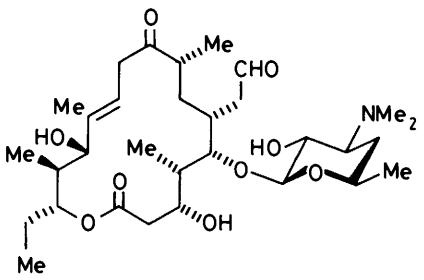


(35) R = CHO
(36) R = $CH(OMe)_2$



(37) R = CHO
(38) R = $CH(OMe)_2$



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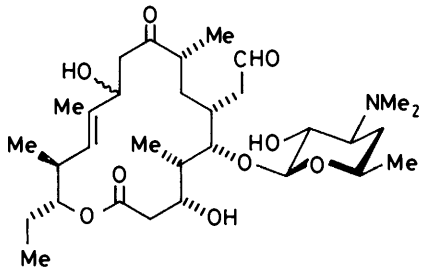

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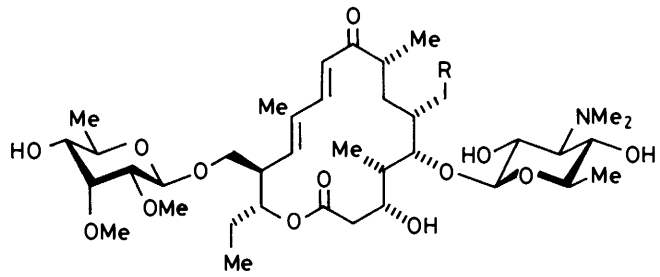
dimethylacetal. Treatment of compound (**58**) with acetic anhydride in methanol afforded a less polar *N*-acetyl derivative (**59**) as a 2:1 mixture of rotamers, lending further support to the fact the molecule contained an –NHMe group. Mild acidic hydrolysis of (**51**) with aqueous DFA afforded 12,13-epoxytylosin (**52**), together with 12,13-epoxydesmycosin (**46**). Even under these carefully controlled and extremely mild acidic conditions, extensive hydrolysis of the mycarose moiety was observed.

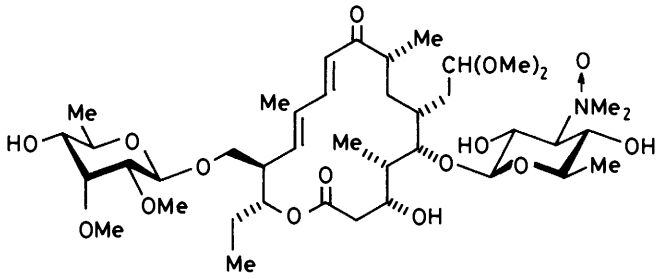
We next turned our attention to the preparation of the 12,13-epoxides of the esterified tylosin and 23-DMT derivatives, in which the esterified mycarose moieties would be expected to be more stable towards mild acidic hydrolysis. 4″-*O*-Isovaleryltylosin (**16**)[1] on treatment with methanol in the presence of either DFA or trifluoroacetic acid (TFA) gave high yields of the desired dimethyl acetal (**48**). The latter reacted with MCPBA to give the epoxide *N*-oxide (**50**), which on heating with triphenylphosphine gave 12,13-epoxy-4″-*O*-isovaleryltylosin 20-dimethylacetal (**53**). Mild acidic hydrolysis of (**53**) with aqueous DFA gave 12,13-epoxy-4″-*O*-isovaleryltylosin (**54**).

Acetylation of compound (**54**) with acetic anhydride and DMAP in the presence of triethylamine gave the 2′,3,4‴-tri-*O*-acetyl derivative (**55**). Hydrolysis of the latter with 2% triethylamine in methanol at 25 °C gave 3-*O*-acetyl-12,13-epoxy-4″-*O*-isovaleryltylosin (**56**), acetylation of which with acetic anhydride in acetone gave the 2′,3-di-*O*-acetate (**57**). Oxidation of compound (**57**) under Pfitzner–Moffatt conditions, followed by hydrolysis with 2% triethylamine in methanol, gave a high yield of 3-*O*-acetyl-23-*O*-demycinosyl-12,13-epoxy-4″-*O*-isovaleryltylosin (**60**). The latter on treatment with acetic anhydride and DMAP in the presence of triethylamine gave the 2′,3,23-tri-*O*-acetyl derivative (**61**).

The 3-*O*-acetyl-4″-*O*-isovaleryltylosin hydrazone derivatives (**22**) and (**23**) exhibited a similar antibacterial spectrum and potency to that of the parent aldehydes,[28,29] and also showed improved activity against macrolide-resistant strains of *Staphylococcus aureus*.[30,31] They exhibited higher serum levels when administered i.v. in mice, but lower serum levels when given p.o. to squirrel monkeys, than the parent aldehydo derivatives.[28,29] The 3-*O*-acetyl-4″-*O*-isovaleryl-23-DMT derivatives and their hydrazones were two- to four-fold more potent than their tylosin analogues, but were inactive against



(41) R = CHO
(42) R = $CH(OMe)_2$



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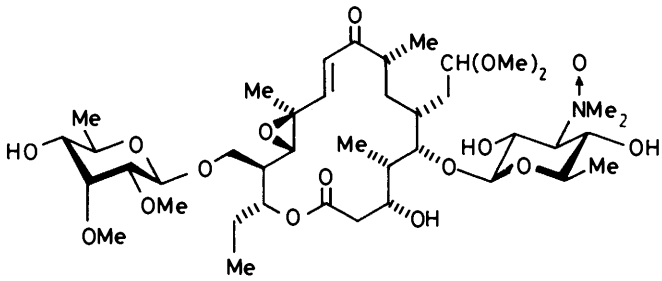

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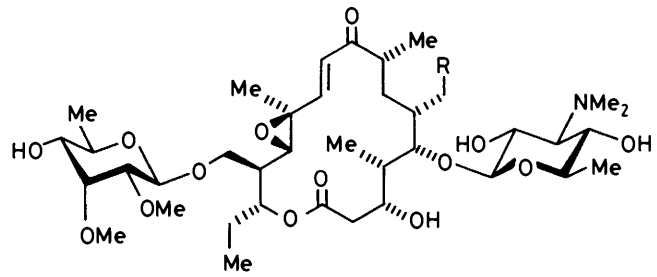


(45) R = $CH(OMe)_2$
(46) R = CHO

macrolide-resistant strains of *Staphylococcus aureus*. Their serum levels, however, were superior to those of tylosin (**8**) or 23-DMT (**1**). The hydrazone derivatives again exhibited higher serum levels than the aldehydes, when administered i.v. in mice, but lower serum levels when given orally in squirrel monkeys. The 23-DMT derivatives (**2**) and (**27**) showed lower serum levels than the corresponding tylosin derivatives (**9**) and (**22**) respectively. However, the triacetyl derivatives (**4**) and (**28**) gave excellent serum levels that were even higher than those observed with (**9**) and (**22**) respectively.[32] In general the 12,13-epoxy derivatives exhibited a similar spectrum and potency to that of the parent dienone macrolides.[32] They were, however, slightly less active against most *Streptococcus* strains with the exception of *pneumoniae*, against which they were more potent than the corresponding dienones. The serum levels of the 12,13-epoxy derivatives were only slightly lower than those of the corresponding dienone macrolides. The 23-DMT derivatives (**4**) and (**61**) exhibited better oral absorption than erythromycin in squirrel monkeys and mice. However, in oral mouse protection studies they were found to be only as effective and no better than erythromycin, against staphylococci and streptococci.[32] They represent the first derivatives of 23-DMT that have good oral serum levels and activity in mouse protection studies and their *in vivo* efficacy is superior to any of the 23-acylderivatives,[33] or 3,4″-diacyl derivatives, of 23-DMT[34] that have been reported to date.

Experimental

Data were recorded as described in the preceding paper.[1] The ^1H and ^{13}C n.m.r. data are given in Supplementary Publication No. SUP 56738 (22 pp.).*

2′,4‴-Di-O-acetyltylosin (**11**).—2′-*O*-Acetyltylosin (**10**)[1] (121.8 g), DMAP (3.11 g), and dry pyridine (15.47 ml) were dissolved in dry dichloromethane (1.5 l) and acetic anhydride (7.18 ml) was added. The mixture was kept at 25 °C for 19 h. Additional acetic anhydride (3.59 ml) was added and the reaction was continued for a total of 47 h. Chromatography [h.p.l.c., 2 cartridges; substrate (40 g)] (25→50% Me_2CO in C_6H_{14}) gave *2′,4‴-di-O-acetyltylosin* (**11**) (53.9 g, 49%) (Found: C, 60.1; H, 8.2; N, 1.3. $C_{50}H_{81}NO_{19}$ requires C, 60.04; H, 8.16; N, 1.40%); $[\alpha]_D^{26}$ −49.8° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 285 nm (21 610); $\nu_{max.}$($CDCl_3$) 3 490, 1 740, 1 680, 1 600, 1 240, 1 168, and 1 058 cm^{-1}; and from the less polar fractions, *2′,4″,4‴-tri-O-acetyltylosin* (**12**) (3.6 g, 3%) (Found: C, 59.9; H, 8.0; N, 1.0. $C_{52}H_{83}NO_{20}$ requires C, 59.93; H, 8.03; N, 1.34%); *m/z* 1 042 (MH^+); $[\alpha]_D^{26}$ −45.8° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 285 nm (21 680); $\nu_{max.}$($CDCl_3$) 3 490, 1 638, 1 675, 1 590, 1 240, 1 160, and 1 050 cm^{-1}. The most polar fractions afforded unchanged monoacetate (**10**) (16.8 g, 16%).

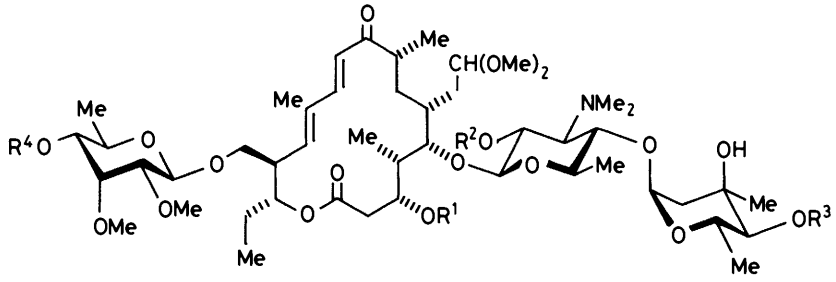
Preparation of Chloroacetyl Monosaccharides.—The sugar (1 mol equiv.) and dry pyridine (*x* mol equiv.) were dissolved in dry dichloromethane (*y* ml) and chloroacetyl chloride (*z* mol equiv.) was added. The mixture was stirred at 0 °C for 4 h.

Methyl β-L-mycaroside (**17**) (730 mg) (*x* = 3.6, *y* = 26, *z* = 3.6) gave, after chromatography (30 × 2.5 cm; 10% EtOAc in CH_2Cl_2), *methyl 4-O-chloroacetyl-β-L-mycaroside* (**18**) (625 mg, 60%) as needles, m.p. 108 °C (Found: C, 47.3; H, 6.7; Cl, 13.8. $C_{10}H_{17}ClO_5$ requires C, 47.53; H, 6.78; Cl, 14.03%); $[\alpha]_D^{26}$ +24.0° ($CHCl_3$); $\nu_{max.}$($CDCl_3$) 3 590, 1 745, 1 160, 1 047, and 1 015 cm^{-1}.

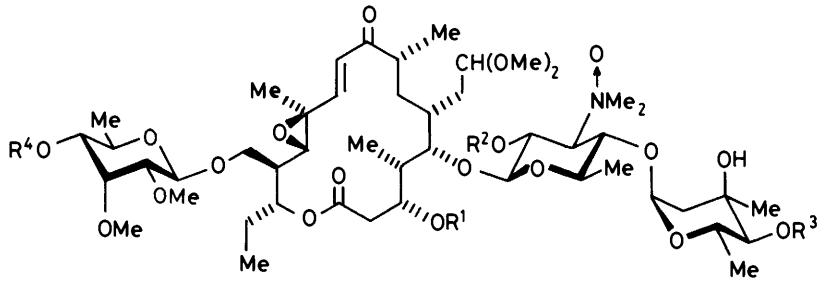
Acetylation of Methyl 4-O-Chloroacetylmycaroside.—The sugar (1 mol equiv.), DMAP (*v* mol equiv.) and triethylamine (*w* ml) were dissolved in dry dichloromethane (*x* ml). A solution of acetic anhydride (*y* mol equiv.) in dry dichloromethane (*z* ml) was added dropwise during 30—45 min to the stirred solution at 25 °C. After 16 h the reaction mixture was worked up.

The chloroacetate (**18**) (300 mg) (*v* = 1, *w* = 0.5, *x* = 20, *y* = 1, *z* = 10) gave, after chromatography (30 × 2.5 cm; 2% EtOAc in CH_2Cl_2), methyl 4-*O*-acetyl-3-*O*-chloroacetyl-β-L-

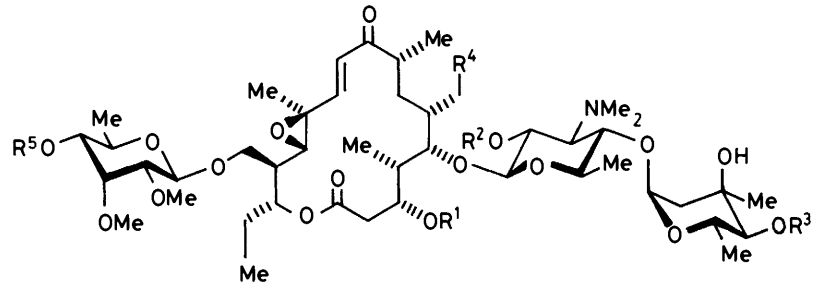
* For details of the Supplementary Publication Scheme, see section 4 of Instructions for Authors, issue 1.



(47) $R^1 = R^2 = R^3 = R^4 = H$
(48) $R^1 = R^2 = R^4 = H$, $R^3 = COCH_2CHMe_2$



(49) $R^1 = R^2 = R^3 = R^4 = H$
(50) $R^1 = R^2 = R^4 = H$, $R^3 = COCH_2CHMe_2$



(51) $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CH(OMe)_2$
(52) $R^1 = R^2 = R^3 = R^5 = H$, $R^4 = CHO$
(53) $R^1 = R^2 = R^5 = H$, $R^3 = COCH_2CHMe_2$, $R^4 = CH(OMe)_2$
(54) $R^1 = R^2 = R^5 = H$, $R^3 = COCH_2CHMe_2$, $R^4 = CHO$
(55) $R^1 = R^2 = R^5 = Ac$, $R^3 = COCH_2CHMe_2$, $R^4 = CHO$
(56) $R^1 = Ac$, $R^2 = R^5 = H$, $R^3 = COCH_2CHMe_2$, $R^4 = CHO$
(57) $R^1 = R^2 = Ac$, $R^5 = H$, $R^3 = COCH_2CHMe_2$, $R^4 = CHO$

mycaroside (**19**) (158 mg, 45%) as a gum, $[\alpha]_D^{26}$ −33.4° ($CHCl_3$); $\nu_{max.}$($CDCl_3$) 1 720, 1 210, 1 127, and 1 030 cm^{-1}.

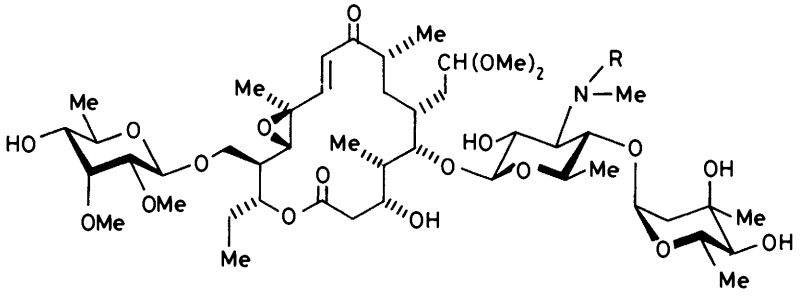
Preparation of Methyl 4-O-*Acetylmycaroside* (**20**).—*Method* 1. The sugar (1 mol equiv.) was dissolved in 5% (v/v) triethylamine in methanol (10 ml) and the solution was stirred at 25 °C for *x* h. The sugar (**19**) (88 mg) (*x* = 18) gave, after chromatography (p.l.c.; silica gel, 20 × 20 cm; 250 μ; 30% EtOAc in CH_2Cl_2), methyl 4-*O*-acetyl-β-L-mycaroside (**20**) (63 mg, 97%) as needles, m.p. 67—69 °C.[2]

Method 2. The sugar (1 mol equiv.), DMAP (*v* mol equiv.), and triethylamine (*w* ml) were dissolved in dry dichloromethane (*x* ml). Acetic anhydride (*y* mol equiv.) in dry dichloromethane (*z* ml) was added at 25 °C during 0.5 h and the reaction was allowed to continue for 16 h.

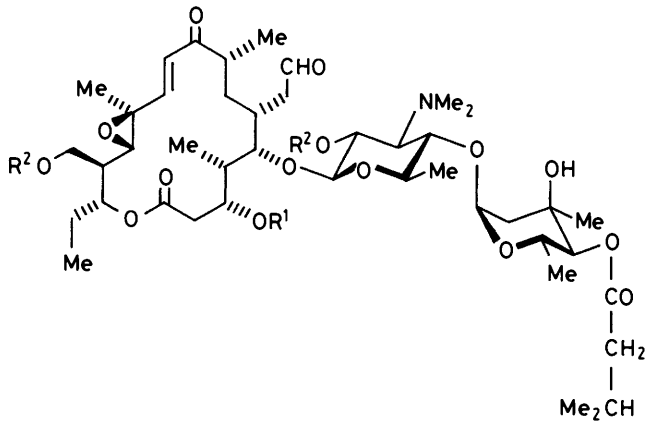
Methyl β-L-mycaroside (**17**) (147 mg) (*v* = 1, *w* = 0.25, *x* = 10, *y* = 1, *z* = 5) gave, after chromatography (p.l.c.; silica gel; 20 × 20 cm; 1 000 μ; 20% EtOAc in CH_2Cl_2), methyl 4-*O*-acetyl-β-L-mycaroside (**20**) (120 mg, 66%) as needles, m.p. 67—69 °C, identical with the previous sample.[2]

General Procedure for the Preparation of 4″-O-*Acyl- and* 3-O-*Acetyl Macrolides.*—The macrolide (1 mol equiv.), DMAP (*u* mol equiv.), and triethylamine (*v* mol equiv.) were dissolved in dry dichloromethane (*w* ml). The acid anhydride (*x* mol equiv.) was added and the mixture was stirred at 25 °C for *y* h.

(a) The macrolide (**11**) (53.8 g) with isovaleric anhydride (*u* = 0.5, *v* = 10, *w* = 1 080, *x* = 1, *y* = 18) gave, after chromatography (h.p.l.c., 2 cartridges; 17% Me_2CO in C_6H_{14}), 2′,4‴-*di*-O-*acetyl*-4″-O-*isovaleryltylosin* (**13**) (28.5 g, 49%) (Found: C, 60.1; H, 7.9; N, 1.05. $C_{55}H_{89}NO_{20}$ requires C, 60.92; H, 8.35; N, 1.28%); *m*/*z* 1 098 (MH^+); $[\alpha]_D^{26}$ −47.2° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 285 nm (20 480); $\nu_{max.}$($CDCl_3$) 3 500, 1 745, 1 730, 1 680, 1 595, 1 245, 1 170, 1 060, and 1 040 cm^{-1}. The unchanged



(58) R = H
(59) R = Ac



(60) R^1 = Ac, R^2 = H
(61) R^1 = R^2 = Ac

starting material (**19**) was recycled twice to give a total yield of the isovalerate (**13**) of 35.6 g (61%). A small amount of unchanged starting material (**11**) (1.74 g, 3%) remained.

(b) The macrolide (**13**) (35.8 g) with acetic anhydride (u = 4, v = 10, w = 1 200, x = 4, y = 21) gave, after chromatography (h.p.l.c., 4 cartridges; 17% Me_2CO in C_6H_{14}), 2′,3,4‴-*tri*-O-*acetyl*-4″-O-*isovaleryltylosin* (**14**) (3.08 g, 82%) (Found: C, 60.9; H, 8.0; N, 1.5. $C_{57}H_{91}NO_{21}$ requires C, 60.78; H, 8.14; N, 1.24%); m/z 1 126 (MH^+); $[\alpha]_D^{26}$ −36.2° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 283 nm (21 420); $\nu_{max.}$($CDCl_3$) 3 500, 1 740, 1 680, 1 595, 1 245, 1 170, 1 060, and 1 035 cm^{-1}.

General Procedure for the Deprotection of the 2′- and 4‴-O-Acyl Groups.—The macrolide (1 mol equiv.) was dissolved in a solution of triethylamine (x mol equiv.) (y% v/v) in methanol and the solution was kept at 25 °C for z h.

(a) The macrolide (**14**) (287 mg) (x = 281, y = 33, z = 66) gave, after chromatography (30 × 2 cm; 20% Me_2CO in C_6H_{14}), 3-*deoxy*-4″-O-*isovaleryl*-3,19-*cyclotylosin* (**21**) (104 mg, 41%) (Found: C, 58.7; H, 7.8; N, 1.2. $C_{51}H_{83}NO_{17}$·0.5$CHCl_3$ requires C, 58.79; H, 8.03; N, 1.35%); m/z 982 (MH^+); $[\alpha]_D^{26}$ −60.5° ($CHCl_3$); $\lambda_{max.}$(MeOH) 278 nm (21 370); $\nu_{max.}$($CDCl_3$) 3 678, 3 495, 1 722, 1 650, 1 167, 1 065, and 1 033 cm^{-1}, and 3-*O*-acetyl-4″-*O*-isovaleryltylosin (**9**) (120 mg, 45%).

(b) The macrolide (**14**) (1.85 g) (x = 15, y = 1.75, z = 67) gave, after chromatography (30 × 2 cm; 25% Me_2CO in C_6H_{14}), 3-O-*acetyl*-4″-O-*isovaleryltylosin* (**9**) (868 mg, 51%) as well as a partially deacylated forecut. The latter (783 mg) was dissolved in methanol (50 ml) containing triethylamine (1 ml) and the solution was kept at 25 °C for 66 h. The product was isolated as above to give *compound* (**9**) (total 1.47 g, 86%) (Found: C, 60.7; H, 8.4; N, 1.3. $C_{53}H_{87}NO_{19}$ requires C, 61.07; H, 8.41; N, 1.34%); m/z 1 042 (MH^+); $[\alpha]_D^{26}$ −38.0° ($CHCl_3$); $\lambda_{max.}$(MeOH) 282 nm (21 610); $\nu_{max.}$($CDCl_3$) 3 500, 1 725, 1 680, 1 598, 1 240, 1 168, and 1 055 cm^{-1}.

(c) The macrolide (**55**) (10.4 g) (x = 20, y = 2, z = 82) gave, after chromatography (h.p.l.c., 1 cartridge; 1.5% MeOH in $CHCl_3$), 3-O-*acetyl*-12,13-*epoxy*-4″-O-*isovaleryltylosin* (**56**) (9.4 g, 84%) (Found: C, 58.8; H, 8.2; N, 1.2. $C_{53}H_{87}NO_{20}$·0.2$CHCl_3$ requires C, 58.82; H, 8.10; N, 1.29%); m/z 1 058 (MH^+); $[\alpha]_D^{26}$ −52.2°($CHCl_3$);$[\theta]_{210}$ +78 590,$[\theta]_{246}$ −36 670,$[\theta]_{303}$ +5 240 (MeOH); $\lambda_{max.}$(MeOH) 237 nm (11 210); $\nu_{max.}$($CDCl_3$) 3 505, 1 730, 1 693, 1 622, 1 163, and 1 053 cm^{-1}.

General Procedure for the Preparation of 2′-O-Acetyl Macrolides.—The macrolide (1 mol equiv.) and acetic anhydride (5 mol equiv.) were dissolved in dry acetone (x ml) and the mixture was kept at 25 °C for y h.

(a) The macrolide (**9**) (5 g) (x = 280, y = 18) gave, after chromatography (30 × 6 cm; 15% Me_2CO in C_6H_{14}), 2′,3-*di*-O-*acetyl*-4″-O-*isovaleryltylosin* (**15**) (4.54 g, 87%) (Found: C, 59.6; H, 8.3; N, 1.1. $C_{55}H_{89}NO_{20}$·0.2$CHCl_3$ requires C, 59.61; H, 8.10; N, 1.26%); m/z 1 084 (MH^+); $[\alpha]_D^{26}$ −50.9° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 285 nm (19 980); $\nu_{max.}$($CDCl_3$) 3 495, 1 730, 1 680, 1 592, 1 238, 1 166, 1 058, and 1 027 cm^{-1}.

(b) The macrolide (**56**) (8.83 g) (x = 530, y = 44) gave, after chromatography (30 × 5 cm; 15% Me_2CO in $CHCl_3$), 2′,3-*di*-O-*acetyl*-12,13-*epoxy*-4″-O-*isovaleryltylosin* (**57**) (7.34 g, 86%) (Found: C, 58.2; H, 7.9; N, 1.0. $C_{55}H_{89}NO_{21}$·0.3$CHCl_3$ requires C, 58.15; H, 7.90; N, 1.24%); m/z 1 100 (MH^+); $[\alpha]_D^{26}$ −62.1° ($CHCl_3$); $[\theta]_{223}$ −82 110 (MeOH); $\lambda_{max.}$(CF_3CH_2OH) 238 nm

(11 590); $\nu_{max.}$($CDCl_3$) 3 510, 1 753, 1 710, 1 635, 1 250, 1 180, 1 070, and 1 040 cm^{-1}.

General Procedure for the Preparation of the Hydrazones.—The macrolide (1 mol equiv.) and *N*-amino-4,4-dioxothiomorpholine (*x* mol equiv.) were dissolved in dry THF (*y* ml) unless otherwise stated and the mixture was kept at 25 °C for 19—21 h.

(a) The macrolide (**9**) (495 mg) in ethanol (*x* = 1, *y* = 20) gave, after chromatography (60 × 2 cm; 25% Me_2CO in C_6H_{14}), the *hydrazone* (**22**) (437 mg, 79%) (Found: C, 58.0; H, 7.95; N, 3.4. $C_{57}H_{95}N_3O_{20}S$ requires C, 58.29; H, 8.15; N, 3.58%); *m/z* 1 174 (MH^+); $[\alpha]_D^{26}$ −87.3° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 235 (7 620) and 285 nm (21 770); $\nu_{max.}$($CDCl_3$) 3 500, 1 738, 1 675, 1 595, 1 195, 1 165, 1 130, and 1 060 cm^{-1}.

(b) The macrolide (**15**) (400 mg) (*x* = 2, *y* = 35) gave, after chromatography (15 × 2 cm; 25% Me_2CO in C_6H_{14}), the hydrazone (**23**) (396 mg, 88%).

(c) The macrolide (**3**) (600 mg) (*x* = 2, *y* = 20) gave, after chromatography (60 × 2 cm; 15% Me_2CO in C_6H_{14}), the hydrazone (**26**) (496 mg, 72%).

(d) The macrolide (**2**) (450 mg) (*x* = 2, *y* = 80) gave, after chromatography (120 × 2 cm; 20% Me_2CO in C_6H_{14}), the hydrazone (**27**) (441 mg, 85%).

(e) The macrolide (**4**) (517 mg) (*x* = 2, *y* = 20) gave, after chromatography (30 × 2 cm; 15% Me_2CO in C_6H_{14}), the *hydrazone* (**28**) (538 mg, 91%) (Found: C, 58.6; H, 7.9; N, 3.7. $C_{53}H_{85}N_3O_{18}S$ requires C, 58.71; H, 7.90; N, 3.88%); *m/z* 1 084 (MH^+); $[\alpha]_D^{26}$ −110.0° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 237 (7 980) and 281 nm (22 230); $\nu_{max.}$($CDCl_3$) 3 490, 1 740, 1 593, 1 312, 1 242, 1 187, 1 177, 1 127, 1 058, and 1 030 cm^{-1}.

(f) The macrolide (**6**) (418 mg) (*x* = 1.2, *y* = 50) gave, after chromatography (15 × 2 cm; 16% Me_2CO in C_6H_{14}), the *hydrazone* (**29**) (416 mg, 89%) (Found: C, 61.0; H, 7.9; N, 3.1. $C_{57}H_{87}N_3O_{17}S$ requires C, 61.21; H, 7.84; N, 3.76%); *m/z* 1 118 (MH^+); $[\alpha]_D^{26}$ −76.8° ($CHCl_3$); $\lambda_{max.}$(MeOH) 241 (7 280) and 281 nm (20 650); $\nu_{max.}$($CDCl_3$) 3 460, 1 730, 1 677, 1 592, 1 305, 1 247, 1 183, 1 163, 1 122, 1 052, and 1 017 cm^{-1}.

(g) The macrolide (**7**) (470 mg) (*x* = 2, *y* = 60) gave, after chromatography (30 × 2 cm; 20% Me_2CO in C_6H_{14}), the *hydrazone* (**30**) (422 mg, 78%) (Found: C, 57.35; H, 7.9; N, 3.9. $C_{51}H_{83}N_3O_{17}S$ requires C, 57.45; H, 7.85; N, 3.94%); *m/z* 1 042 (MH^+); $[\alpha]_D^{26}$ −101.9° ($CHCl_3$); $\lambda_{max.}$(MeOH) 238 (10 300) and 280 nm (21 740); $\nu_{max.}$($CDCl_3$) 3 435, 1 728, 1 672, 1 588, 1 305, 1 242, 1 180, 1 165, 1 120, 1 050, and 1 023 cm^{-1}.

Preparation of 23-O-*Demycinosyltylosin Derivatives by Pfitzner–Moffatt Oxidation.*—The macrolide (1 mol equiv.) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodi-imide hydrochloride (12 mol equiv.) were dissolved in dry dimethyl sulphoxide (DMSO) (*x* ml). A solution of pyridine (4 mol equiv.) and TFA (2 mol equiv.) in dry DMSO (*y* ml) was added and the mixture was stirred at 25 °C for *z* h. The mixture was worked up and converted into the 23-*O*-demycinosyl derivative as outlined below.

(a) The macrolide (**15**) (500 mg) (*x* = 12.5, *y* = 2.2, *z* = 18) gave, after chromatography (30 × 2 cm; 13% Me_2CO in C_6H_{14}) using rapid elution, 2',3-*di*-O-*acetyl*-4'''-*deoxy*-4''-O-*isovaleryl*-4'''-*oxotylosin* (**24**) (307 mg, 61%) (Found: C, 60.0; H, 8.7; N, 1.1. $C_{55}H_{87}NO_{20}$·0.1$CHCl_3$ requires C, 60.37; H, 8.02; N, 1.28%); *m/z* 1 082 (MH^+); $[\alpha]_D^{26}$ −38.8° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 284 nm (22 100); $\nu_{max.}$($CDCl_3$) 3 680, 3 500, 1 734, 1 675, 1 594, 1 238, 1 165, 1 060, and 1 027 cm^{-1}.

The ketone (**24**) (5 mg) was dissolved in a 1.3% (v/v) solution of triethylamine in methanol (0.013 ml in 1 ml) and the mixture was stirred at 25 °C for 20 h to give 2',3-di-*O*-acetyl-23-*O*-demycinosyl-4''-*O*-isovaleryltylosin (**3**) (50%) and 3-*O*-acetyl-23-*O*-demycinosyl-4''-*O*-isovaleryltylosin (**2**) (45%).

(b) The macrolide (**15**) (750 mg) (*x* = 20, *y* = 1, *z* = 19) gave, after chromatography (30 × 2 cm; 0→13% Me_2CO in $CHCl_3$ and 30 × 2 cm; 8% Me_2CO in $CHCl_3$), 2',3-*di*-O-*acetyl*-2''',3'''-*didehydro*-2'''-*demethoxy*-4'''-*deoxy*-4''-O-*isovaleryl*-4'''-*oxotylosin* (**25**) (267 mg, 37%) (Found: C, 59.6; H, 7.4; N, 1.2. $C_{54}H_{83}NO_{19}$·0.3$CHCl_3$ requires C, 59.72; H, 7.70; N, 1.29%); *m/z* 1 051 (MH^+); $[\alpha]_D^{26}$ −29.7° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 283 nm (21 850); $\nu_{max.}$($CDCl_3$) 3 680, 3 498, 1 735, 1 678, 1 628, 1 596, 1 238, 1 167, 1 060, and 1 028 cm^{-1}; and 2',3-*di*-O-*acetyl*-23-O-*demycinosyl*-4''-O-*isovaleryltylosin* (**3**) (159 mg, 25%) (Found: C, 56.4; H, 7.0; N, 1.2. $C_{47}H_{75}NO_{16}$·0.8$CHCl_3$ requires C, 56.24; H, 7.52; N, 1.39%); *m/z* 910 (MH^+); $[\alpha]_D^{26}$ −40.6° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 284 nm (16 890); $\nu_{max.}$($CDCl_3$) 3 490, 1 737, 1 680, 1 595, 1 238, 1 167, 1 059, and 1 029 cm^{-1}.

The ketone (**25**) (104 mg) was dissolved in a 1.73% (v/v) solution of triethylamine in methanol (0.277 ml in 16 ml) and the solution was kept at 25 °C for 18 h. Chromatography (30 × 2 cm; 15% Me_2CO in $CHCl_3$) gave 2',3-di-*O*-acetyl-23-*O*-demycinosyl-4''-*O*-isovaleryltylosin (**3**) (24 mg, 26%) and 3-O-*acetyl*-23-O-*demycinosyl*-4''-O-*isovaleryltylosin* (**2**)[15] (50 mg, 58%) (Found: C, 61.5; H, 8.15; N, 2.1. $C_{45}H_{73}NO_{15}$·0.1$CHCl_3$ requires C, 61.42; H, 8.36; N, 1.59%); *m/z* 868 (MH^+); $[\alpha]_D^{26}$ −30.7° ($CHCl_3$); $\lambda_{max.}$(MeOH) 282 nm (20 060); $\nu_{max.}$($CDCl_3$) 3 590, 3 480, 1 729, 1 678, 1 596, 1 240, 1 182, 1 173, 1 050, and 1 025 cm^{-1}.

(c) The macrolide (**15**) (2 g) (*x* = 53, *y* = 2, *z* = 36) gave, after chromatography (120 × 2 cm; 8% Me_2CO in CH_2Cl_2), 2',3-di-*O*-acetyl-23-*O*-demycinosyl-4''-*O*-isovaleryltylosin (**3**) (1.21 g, 70%).

(d) The macrolide (**23**) (396 mg) (*x* = 10, *y* = 0.8, *z* = 19) gave a crude product, which was taken up in methanol (50 ml) containing triethylamine (2 ml) and the solution was stirred at 25 °C for 25 h. Chromatography (30 × 2 cm; 22% Me_2CO in C_6H_{14}) gave 2',3-*di*-O-*acetyl*-23-O-*demycinosyl*-20-*deoxo*-20-[(4,4-*dioxothiomorpholino*)*imino*]-4''-O-*isovaleryltylosin* (**26**) (97 mg, 29%) (Found: C, 58.7; H, 7.1; N, 4.7; S, 3.1. $C_{51}H_{83}N_3O_{17}S$ requires C, 58.77; H, 8.03; N, 4.03; S, 3.08%); *m/z* 1 042 (MH^+); $[\alpha]_D^{26}$ −93.7° ($CHCl_3$); $\lambda_{max.}$(MeOH) 240 (7 830), and 282 nm (20 990); $\nu_{max.}$($CDCl_3$) 3 600, 3 490, 1 736, 1 675, 1 592, 1 240, 1 185, 1 176, 1 122, 1 050, and 1 026 cm^{-1}; and 3-O-*acetyl*-23-O-*demycinosyl*-20-*deoxo*-20-[(4,4-*dioxothiomorpholino*)*imino*]-4''-O-*isovaleryltylosin* (**27**) (47 mg, 15%) (Found: C, 57.6; H, 7.65; N, 4.1; S, 3.5. $C_{49}H_{81}N_3O_{16}S$·0.2$CHCl_3$ requires C, 57.47; H, 7.97; N, 4.10; S, 3.13%); *m/z* 1 000 (MH^+); $[\alpha]_D^{26}$ −85.8° ($CHCl_3$); $\lambda_{max.}$(MeOH) 239 (8 200) and 282 nm (20 710); $\nu_{max.}$($CDCl_3$) 3 600, 3 482, 1 733, 1 675, 1 591, 1 308, 1 250, 1 185, 1 175, 1 122, 1 050, and 1 027 cm^{-1}.

(e) The macrolide (**57**) (500 mg) (*x* = 1.66, *y* = 0.657, *z* = 19) gave a crude product which was taken up in methanol (70 ml) containing triethylamine (1.412 ml) and the solution was stirred at 25 °C for 61 h. Chromatography (15 × 5 cm; 10% Me_2CO in $CHCl_3$) gave 3-O-*acetyl*-23-O-*demycinosyl*-12,13-*epoxy*-4''-O-*isovaleryltylosin* (**60**) (264 mg, 66%) (Found: C, 61.0; H, 8.3; N, 1.3. $C_{45}H_{73}NO_{16}$ requires C, 61.14; H, 8.32; N, 1.58%); *m/z* 884 (MH^+); $[\alpha]_D^{26}$ −50.4° ($CHCl_3$); $[\theta]_{218}$ + 62 850, $[\theta]_{247}$ −20 950, $[\theta]_{281}$ +6 285 (MeOH); $\lambda_{max.}$(MeOH) 238 nm (10 290); $\nu_{max.}$($CDCl_3$) 3 580, 3 480, 1 725, 1 690, 1 620, 1 236, 1 160, 1 115, 1 050, and 1 022 cm^{-1}.

Preparation of 23-O-*Acyl Macrolides.*—*Method* 1. The macrolide (1 mol equiv.), DMAP (0.2 mol equiv.) pyridine (10 mol equiv.), and acetic anhydride (5 mol equiv.) were dissolved in dry dichloromethane (*x* ml) and the mixture was stirred at 25 °C for *y* h.

(a) The macrolide (**2**) (1.2 g) (*x* = 200, *y* = 43) gave, after chromatography (30 × 2 cm; 15% Me_2CO in C_6H_{14}), 2',3,23-*tri*-O-*acetyl*-23-O-*demycinosyl*-4''-O-*isovaleryltylosin* (**4**) (1.08 g, 82%) (Found: C, 61.7; H, 8.25; N, 1.0. $C_{49}H_{77}NO_{17}$ requires

C, 61.81; H, 8.15; N, 1.47%); *m/z* 952 (MH^+); $[\alpha]_D^{26}$ −47.5° ($CHCl_3$); $\lambda_{max.}$(CF_3CH_2OH) 282 nm (21 504); $\nu_{max.}$($CDCl_3$) 3 470, 1 730, 1 595, 1 240, 1 166, 1 120, 1 058, and 1 028 cm^{-1}.

(b) The macrolide (**3**) (550 mg) (*x* = 80, *y* = 20) gave, after chromatography (60 × 2 cm; 15% Me_2CO in C_6H_{14}), 2′,3,23-tri-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**4**) (548 mg, 95%), *m/z* 952 (MH^+).

Method 2. The macrolide (**3**) (599 mg), pyridine (0.293 ml), and phenylacetyl chloride (0.1917 ml) were dissolved in dry dichloromethane (15 ml) and the mixture was kept at −7 °C for 20 h. Additional phenylacetyl chloride (0.1150 ml) was added and the reaction was continued for a total of 41 h. Chromatography (60 × 2 cm; 15% Me_2CO in C_6H_{14}) gave 2′,3-di-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryl-23-*O*-phenylacetyltylosin (**5**) (604 mg, 89%), *m/z* 1 029 (MH^+).

General Procedure for the Methanolysis of the 2′-O-*Acetyl Group.*—The macrolide (1 mol equiv.) was dissolved in methanol (*x* ml and the solution was heated at *y* °C for *z* h.

(a) The macrolide (**5**) (581 mg) (*x* = 70, *y* = 50, *z* = 7) gave, after chromatography (15 × 2.5 cm; 17% Me_2CO in C_6H_{14}), 3-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryl-23-*O*-phenylacetyltylosin (**6**) (500 mg, 90%).

(b) The macrolide (**3**) (944 mg) (*x* = 120, *y* = 40, *z* = 40) gave, after chromatography (60 × 2 cm; 15% Me_2CO in C_6H_{14}), 3-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**2**) (894 mg, 91%).

(c) The macrolide (**4**) (548 mg) (*x* = 70, *y* = 40, *z* = 9) gave, after chromatography (60 × 2 cm; 18% Me_2CO in C_6H_{14}), 3,23-di-*O*-acetyl-23-*O*-demycinosyl-4″-*O*-isovaleryltylosin (**7**) (470 mg, 82%).

Preparation of 20-*Dimethyl Acetals of Macrolides.*—The macrolide (1 mol equiv.) and the fluoroacetic acid (25 mol equiv.) were dissolved in methanol (*x* ml) and the mixture was kept at 25 °C for *y* h. Triethylamine (*z* ml) was added and after 10—15 min the reaction mixture was worked up in the usual way.

(a) The macrolide (**37**) (5 g) and DFA (*x* = 1 000, *y* = 120, *z* = 52) gave, after chromatography (30 × 2.5 cm; 2% MeOH in $CHCl_3$), 12,13-*de-epoxy*-12,13-*didehydrorosamicin* 20-*dimethyl acetal* (**38**) (4.10 g, 76%) (Found: C, 64.3; H, 9.3; N, 2.2. $C_{33}H_{57}NO_9$ requires C, 64.78; H, 9.39; N, 2.29%); *m/z* 612 (MH^+); $[\alpha]_D^{26}$ −4.7° ($CHCl_3$); $[\theta]_{225}$ −40 650, $[\theta]_{225}$ +4 065, $[\theta]_{293}$ −52 030, $[\theta]_{318}$ −27 640, $[\theta]_{342}$ −37 400 (MeOH); $\lambda_{max.}$(MeOH) 282 nm (22 400); $\nu_{max.}$($CDCl_3$) 3 430, 1 710, 1 672, 1 590, 1 315, 1 285, 1 185, 1 180, 1 110, and 1 050 cm^{-1}.

(b) The macrolide (**8**) (5.7 g) and DFA (*x* = 714, *y* = 67, *z* = 27) gave, after chromatography (30 × 5 cm; 2% MeOH in $CHCl_3$), *tylosin* 20-*dimethyl acetal* (**47**) (4.64 g, 78%) (Found: C, 57.65; H, 8.15; N, 1.3. $C_{48}H_{83}NO_{18}$·0.3$CHCl_3$ requires C, 57.77; H, 8.38; N, 1.40%); *m/z* 962 (MH^+); $[\alpha]_D^{26}$ −33.3° ($CHCl_3$); $[\theta]_{226}$ +465, $[\theta]_{278}$ +102 260 (MeOH); $\lambda_{max.}$(MeOH) 282 nm (20 840); $\nu_{max.}$($CDCl_3$) 3 530, 3 460, 1 721, 1 674, 1 590, 1 315, 1 158, and 1 050 cm^{-1}; and desmycosin 20-dimethyl acetal (**42**) (1.08 g, 21%).

(c) The macrolide (**16**)[1] (5 g) and DFA (*x* = 600, *y* = 90, *z* = 30) gave, after chromatography (60 × 2.5 cm; 1% MeOH in $CHCl_3$), 4″-O-*isovaleryltylosin* 20-*dimethyl acetal* (**48**) (4.5 g, 86%) (Found: C, 59.2; H, 8.4; N, 1.2. $C_{53}H_{91}NO_{19}$·0.2$CHCl_3$ requires C, 59.48; H, 8.57; N, 1.31%); *m/z* 1 046 (MH^+); $[\alpha]_D^{26}$ −60.3° ($CHCl_3$); $\lambda_{max.}$(MeOH) 282 nm (21 900); $\nu_{max.}$($CDCl_3$) 3 480, 1 725, 1 672, 1 590, 1 315, 1 170, 1 120, and 1 050 cm^{-1}.

(d) The macrolide (**16**)[1] (200 mg) and TFA (*x* = 2, *y* = 92, *z* = 2) gave, after chromatography (10 × 2.5 cm; 1% MeOH in $CHCl_3$), 4″-*O*-isovaleryltylosin 20-dimethyl acetal (**48**) (187 mg, 72%).

Preparation of Macrolide 3′-N-*Oxides.*—The macrolide (1 mol equiv.) and MCPBA (2 mol equiv.) were dissolved in dry dichloromethane (*x* ml) and the mixture was kept at 25 °C for 1 h.

(a) Rosaramicin (**34**) (2 g) (*x* = 935) gave, after chromatography (60 × 2 cm; 5% MeOH in $CHCl_3$), the N-*oxide* (**35**) (1.53 g, 74%).

(b) The macrolide (**42**)[27] (500 mg) (*x* = 166) gave, after chromatography (30 × 2 cm; 3% MeOH in $CHCl_3$), the N-*oxide* (**43**) (318 mg, 31%) (Found: C, 57.3; H, 7.8; N, 1.7. Calc. for $C_{41}H_{71}NO_{16}$·0.2$CHCl_3$: C, 57.40; H, 8.34; N, 1.63%); *m/z* 834 (MH^+); $[\alpha]_D^{26}$ −1.0° ($CHCl_3$); $[\theta]_{223}$ −40 330, $[\theta]_{275}$ +55 450, $[\theta]_{347}$ −20 160 (MeOH); $\lambda_{max.}$(MeOH) 282 nm (21 030); $\nu_{max.}$($CDCl_3$) 3 540, 1 706, 1 672, 1 590, 1 184, 1 164, 1 125, and 1 065 cm^{-1}.

Preparation of 12,13-*Epoxy Macrolides.*—The macrolide (1 mol equiv.), MCPBA (*u* mol equiv.), and disodium hydrogen phosphate (*v* mol equiv.) were added to dry dichloromethane (*w* ml) and the mixture was stirred at *x* °C for *y* h.

(a) Mycinamicin V (**31**)[19,20] (2 g) (*u* = 12, *v* = 20, *w* = 100, *x* = 60, *y* = 23) gave, after chromatography (30 × 2.5 cm; 2→4% MeOH in $CHCl_3$), *mycinamicin* II 3′-N-*oxide* (**32**) (811 mg, 39%) (Found: C, 55.7; H, 7.2; N, 1.6. $C_{37}H_{61}NO_{14}$·0.5$CHCl_3$ requires C, 55.30; H, 7.65; N, 1.74%); *m/z* 744 (MH^+); $[\alpha]_D^{26}$ −17.6° ($CHCl_3$); $[\theta]_{237}$ −174 350 (MeOH); $\lambda_{max.}$(MeOH) 215sh nm (24 860); $\nu_{max.}$($CDCl_3$) 3 540, 3 400, 1 709, 1 648, 1 620, 1 332, 1 177, 1 123, 1 112, 1 083, and 1 063 cm^{-1}.

(b) The macrolide (**38**) (2 g) (*u* = 10, *v* = 0, *w* = 120, *x* = 25, *y* = 19) gave, after chromatography (90 × 2 cm; 3% MeOH in $CHCl_3$), *rosaramicin* 20-*dimethyl acetal* 3′-N-*oxide* (**36**) (1.43 g, 68%) (Found: C, 57.5; H, 8.1; N, 1.7. $C_{33}H_{57}NO_{11}$·0.4$CHCl_3$ requires C, 57.31; H, 8.31; N, 2.03%); *m/z* 644 (MH^+); $[\alpha]_D^{26}$ −12.1° ($CHCl_3$); $[\theta]_{242}$ −119 290, $[\theta]_{285}$ −1 630, $[\theta]_{340}$ −7 350 (MeOH); $\lambda_{max.}$(MeOH) 236 nm (11 720); $\nu_{max.}$($CDCl_3$) 3 525, 1 712, 1 690, 1 618, 1 317, 1 186, 1 115, and 1 063 cm^{-1}.

(c) The macrolide (**38**) (1 g) (*u* = 10, *v* = 17, *w* = 60, *x* = 25, *y* = 63) gave, after chromatography (60 × 2 cm; 3% MeOH in $CHCl_3$), the *N*-oxide (**36**) (557 mg, 53%).

(d) The macrolide (**42**)[27] (3 g) (*u* = 6, *v* = 10, *w* = 150, *x* = 60, *y* = 23) gave, after chromatography (60 × 2.5 cm; 2% MeOH in $CHCl_3$), 12,13-*epoxydesmycosin* 20-*dimethyl acetal* 3′-N-*oxide* (**44**) (1.42 g, 46%) (Found: C, 55.3; H, 7.95; N, 1.5. $C_{41}H_{71}NO_{17}$·0.3$CHCl_3$ requires C, 55.59; H, 8.08; N, 1.58%); *m/z* 850 (MH^+); $[\alpha]_D^{26}$ −12.4° ($CHCl_3$); $[\theta]_{243}$ −34 740 (MeOH); $\lambda_{max.}$(MeOH) 237 nm (11 980); $\nu_{max.}$($CDCl_3$) 3 570, 3 550, 3 360, 1 720, 1 692, 1 620, 1 218, 1 170, 1 128, 1 080, and 1 070 cm^{-1}. Traces of desmycosin 20-dimethylacetal 3′-*N*-oxide (**43**) (36 mg, 1%) were also isolated.

(e) The macrolide (**42**)[27] (500 mg) (*u* = 6, *v* = 0, *w* = 25, *x* = 60, *y* = 23) gave, after chromatography (30 × 2.5 cm; 5% MeOH in $CHCl_3$), the epoxide (**44**) (180 mg, 37%).

(f) The macrolide (**42**)[27] (200 mg) (*u* = 10, *v* = 17, *w* = 75, *x* = 25, *y* = 8) gave, after chromatography (15 × 2.5 cm; 1.5% MeOH in $CHCl_3$), the epoxide (**44**) (110 mg, 53%).

(g) The macrolide (**47**) (1.5 g) (*u* = 10, *v* = 17, *w* = 75, *x* = 25, *y* = 8) gave, after chromatography (30 × 2.5 cm; 0→8% MeOH in $CHCl_3$), 12,13-*epoxytylosin* 20-*dimethyl acetal* 3′-N-*oxide* (**49**) (1.11 g, 72%) (Found: C, 55.9; H, 7.2; N, 1.2. $C_{48}H_{83}NO_{20}$·0.3$CHCl_3$ requires C, 55.97; H, 8.12; N, 1.36%); *m/z* 994 (MH^+); $[\alpha]_D^{26}$ −51.4° (MeOH); $[\theta]_{243}$ −45 191°, $[\theta]_{275}$ +2 520, $[\theta]_{340}$ −8 810 (MeOH); $\lambda_{max.}$(MeOH) 236 nm (12 370), $\nu_{max.}$($CDCl_3$) 3 535, 1 718, 1 690, 1 618, 1 315, 1 162, 1 127, and 1 057 cm^{-1}.

(h) The macrolide (**48**) (3.18 g) (*u* = 10, *v* = 0, *w* = 159, *x* = 25, *y* = 4) gave, after chromatography (30 × 5 cm; 4% MeOH in $CHCl_3$), 12,13-*epoxy*-4″-O-*isovaleryltylosin* 20-*dimethyl acetal* 3′-N-*oxide* (**50**) (2.17 g, 72%) (Found: C, 56.8; H,

8.3; N, 1.1. $C_{53}H_{91}NO_{21}$·0.3$CHCl_3$ requires C, 57.14; H, 8.23; N, 1.26%); *m/z* 1 078 (MH^+); $[\alpha]_D^{26}$ −50.6° ($CHCl_3$); $[\theta]_{245}$ −42 690, $[\theta]_{275}$ +2 440, $[\theta]_{335}$ −9 760 (MeOH); $\lambda_{max.}$(MeOH) 238 nm (13 180); $\nu_{max.}$($CDCl_3$) 3 675, 3 543, 1 725, 1 687, 1 615, 1 163, 1 123, and 1 058 cm^{-1}.

Procedures for the Deprotection of the 3′-N-*Oxides.*—*Method* 1. The macrolide (1 mol equiv.) and triphenylphosphine (*w* mol equiv.) were dissolved in dry dichloromethane (*x* ml) and the mixture was kept *y* °C for *z* h.

(a) The macrolide (**32**) (485 mg) (*w* = 10, *x* = 30, *y* = 55, *z* = 67) gave, after chromatography (30 × 2.5 cm; 4% MeOH in $CHCl_3$), mycinamicin II (**33**)[19,20] (265 mg, 56%) (Found: C, 59.8; H, 8.35; N, 1.8. Calc. for $C_{37}H_{61}NO_{13}$·0.1$CHCl_3$: C, 60.07; H, 8.31; N, 1.89%); *m/z* 728 (MH^+); $[\alpha]_D^{26}$ −2.2° ($CHCl_3$); $[\theta]_{239}$ −147 930 (MeOH); $\lambda_{max.}$(MeOH) 216 nm (21 050); $\nu_{max.}$($CDCl_3$) 3 545, 3 430, 1 710, 1 650, 1 626, 1 333, 1 239, 1 170, 1 070, and 1 055 cm^{-1}.

(b) The macrolide (**35**) (300 mg) (*w* = 10, *x* = 20, *y* = 55, *z* = 71) gave, after chromatography (30 × 2.5 cm; 4% MeOH in $CHCl_3$), rosaramicin (**34**) (206 mg, 71%).

(c) The macrolide (**44**) (350 mg) (*w* = 10, *x* = 20, *y* = 55, *z* = 67) gave, after chromatography (30 × 2.5 cm; 2% MeOH in $CHCl_3$), (12,13-*epoxydesmycosin* 20-*dimethyl acetal* (**45**) (211 mg, 62%) (Found: C, 57.9; H, 8.2; N, 1.6. $C_{41}H_{71}NO_{16}$·0.2$CHCl_3$ requires C, 57.40; H, 8.34; N, 1.63%); *m/z* 834 (MH^+); $[\alpha]_D^{26}$ −18.0° ($CHCl_3$); $[\theta]_{245}$ −54 100, $[\theta]_{285}$ +2 250, $[\theta]_{335}$ −9 020 (MeOH); $\lambda_{max.}$(MeOH) 237 nm (13 840); $\nu_{max.}$($CDCl_3$) 3 540, 3 440, 1 717, 1 688, 1 617, 1 313, 1 187, 1 173, 1 121, and 1 060 cm^{-1}.

(d) The macrolide (**49**) (675 mg) (*w* = 10, *x* = 300, *y* = 25, *z* = 49) gave, after chromatography (30 × 2 cm; 3% MeOH in $CHCl_3$), 12,13-*epoxytylosin* 20-*dimethyl acetal* (**51**) (552 mg, 83%) (Found: C, 55.4; H, 7.5; N, 1.2. $C_{48}H_{83}NO_{19}$·0.5$CHCl_3$ requires C, 55.55; H, 8.06; N, 1.35%); *m/z* 978 (MH^+); $[\alpha]_D^{26}$ −52.7° (MeOH); $[\theta]_{246}$ −42 430, $[\theta]_{275}$ +4 710, $[\theta]_{340}$ −9 430 (MeOH); $\lambda_{max.}$(MeOH) 237 nm (13 760); $\nu_{max.}$($CDCl_3$) 3 543, 3 480, 1 720, 1 692, 1 620, 1 318, 1 190, 1 162, 1 128, and 1 053 cm^{-1}.

(e) The macrolide (**49**) (675 mg) (*w* = 20, *x* = 300, *y* = 25, *z* = 12) gave, after chromatography (30 × 2 cm; 0→8% MeOH in $CHCl_3$), 12,13-epoxytylosin 20-dimethyl acetal (**51**) (487 mg, 73%) and 3′-N-*demethyl*-12,13-*epoxytylosin* 20-*dimethyl acetal* (**58**) (111 mg, 17%) (Found: C, 56.6; H, 7.9; N, 1.3. $C_{47}H_{81}NO_{19}$·0.3$CHCl_3$ requires C, 56.45; H, 8.17; N, 1.40%); *m/z* 964 (MH^+); $[\theta]_{243}$ −51 520, $[\theta]_{280}$ 0, $[\theta]_{335}$ −9 810 (MeOH); $\lambda_{max.}$(MeOH) 238 nm (13 520); $\nu_{max.}$($CDCl_3$) 3 520, 1 718, 1 690, 1 618, 1 313, 1 160, 1 120, and 1 055 cm^{-1}.

(f) The macrolide (**50**) (0.5 g) (*w* = 10, *x* = 205, *y* = 25, *z* = 71) gave, after chromatography (60 × 2 cm; 1.25% MeOH in $CHCl_3$), 12,13-*epoxy*-4″-O-*isovaleryltylosin* 20-*dimethyl acetal* (**53**) (328 mg, 67%) (Found: C, 57.95; H, 8.35; N, 1.2. $C_{53}H_{91}NO_{20}$·0.3$CHCl_3$ requires C, 57.97; H, 8.36; N, 1.28%); *m/z* 1 062 (MH^+); $[\alpha]_D^{26}$ −46.9° ($CHCl_3$); $[\theta]_{248}$ −7 860, $[\theta]_{275}$ +15 720, $[\theta]_{335}$ −7 860 (MeOH); $\lambda_{max.}$(MeOH) 237 nm (12 260); $\nu_{max.}$($CDCl_3$) 3 675, 3 500, 1 722, 1 618, 1 316, 1 186, 1 160, 1 118, and 1 052 cm^{-1}, 12,13-epoxy-4″-*O*-isovaleryltylosin (**54**) (55 mg, 12%), and unchanged 12,13-epoxy-4″-*O*-isovaleryltylosin 20-dimethyl acetal 3′-*N*-oxide (**50**) (9 mg, 2%).

Method 2. The macrolide (**35**) (100 mg) was dissolved in dry dichloromethane (49.9 ml) and carbon disulphide (1.27 g) was added. The mixture was heated in a sealed bomb at 60 °C for 24 h. Methanol (100 ml) was added and after 10 min the reaction mixture was worked up. Chromatography (30 × 1 cm; 2.5% MeOH in $CHCl_3$) gave rosaramicin (**34**) (61 mg, 62%).

De-epoxidation Procedures.—The macrolide (1 mol equiv.) was dissolved in the appropriate solvent. A stock solution of 20% (w/v) titanium(III) chloride* (*x* mol equiv.) in water was added rapidly at 0 °C, and the mixture was stirred for *y* h. The pH of the reaction mixture was *z*.

(a) The macrolide (**34**) (1 g) in 0.1M hydrochloric acid (30 ml) (*x* = 5, *y* = 3, *z* = 1.3) gave, after chromatography (30 × 2.5 cm; 3% MeOH in $CHCl_3$), 12,13-de-epoxyrosaramicin (**37**) (598 mg, 61%).

(b) The macrolide (**35**) (200 mg) in methanol (6 ml) (*x* = 4, *y* = 0.33, *z* = 1.5) gave, after chromatography (30 × 1 cm; 3% MeOH in $CHCl_3$), a 60:40 mixture of the de-epoxyrosaramicin (**37**) and its 20-dimethyl acetal (**38**) (74 mg).

(c) Rosaramicin (**34**) (1 g) in methanol (30 ml) (*x* = 5.7, *y* = 3, *z* = 1.0) gave, after chromatography (60 × 2.5 cm; 5% MeOH in $CHCl_3$), a 1:9 mixture of the de-epoxyrosaramicin (**37**) and its 20-dimethyl acetal (**38**) (744 mg).

(d) Rosaramicin (**34**) (2.51 g) in THF (90 ml) was treated with a solution of disodium hydrogen phosphate (7.33 g) in water (60 ml) (*x* = 5, *y* = 3, *z* = 5.5) to give, after chromatography (60 × 2.5 cm; 3% MeOH in $CHCl_3$), unchanged rosaramicin (**34**) (1.71 g, 68%), 12,13-*de-epoxy*-11,12-*didehydro*-10,11-*dihydro*-13-*hydroxyrosaramicin* (**39**) (504 mg, 20%) (Found: C, 62.2; H, 8.5; N, 2.2. $C_{31}H_{53}NO_9$·0.1$CHCl_3$ requires C, 62.50; H, 8.97; N, 2.35%); *m/z* 584 (MH^+), $[\alpha]_D^{26}$ −26.3° ($CHCl_3$); $[\theta]_{235}$ −5 370, $[\theta]_{300}$ +1 430, $[\theta]_{340}$ −2 690 (MeOH); $\lambda_{max.}$(MeOH) end absorption only; $\nu_{max.}$($CDCl_3$) 3 585, 3 470, 1 710, 1 317, 1 190, 1 104, and 1 048 cm^{-1}, and 12,13-*de-epoxy*-12,13-*didehydro*-10,11-*dihydro*-11-*hydroxyrosaramicin* (**40**) (55 mg, 2%) (Found: C, 61.3; H, 8.6; N, 2.0. $C_{31}H_{53}NO_9$·0.2$CHCl_3$ requires C, 61.28; H, 8.79; N, 2.31%); *m/z* 584 (MH^+); $[\alpha]_D^{26}$ +12.0° ($CHCl_3$); $\lambda_{max.}$(MeOH) end absorption only; $\nu_{max.}$($CDCl_3$) 3 600, 3 430, 1 713, 1 324, 1 160, and 1 045 cm^{-1}.

Procedure for Hydrolysis of the 20-*Dimethyl Acetals.*—*Method* 1. The macrolide (1 mol equiv.) was dissolved in 0.1M aqueous hydrochloric acid (*x* ml) and the solution was kept at 25 °C for *y* h. The solution was poured into a mixture of dichloromethane and water at pH 10—11 and worked up as usual.

(a) The macrolide (**36**) (250 mg) (*x* = 55, *y* = 0.75) gave, after chromatography (15 × 2 cm; 5% MeOH in $CHCl_3$), *rosaramicin* 3′-N-*oxide* (**35**) (174 mg, 75%) (Found: C, 57.4; H, 7.65; N, 1.9. $C_{31}H_{51}NO_{10}$·0.4$CHCl_3$ requires C, 57.68; H, 7.96; N, 2.17%); *m/z* 598 (MH^+); $[\alpha]_D^{26}$ −19.8° ($CHCl_3$); $[\theta]_{242}$ −97 140, $[\theta]_{280}$ −1 500, $[\theta]_{330}$ −4 480 (MeOH); $\lambda_{max.}$(MeOH) 238 nm (12 030); $\nu_{max.}$($CDCl_3$) 3 530, 1 713, 1 692, 1 618, 1 318, 1 186, 1 170, 1 115, and 1 062 cm^{-1}.

(b) The macrolide (**45**) (617 mg) (*x* = 75, *y* = 16) gave, after chromatography (30 × 2.5 cm; 2% MeOH in $CHCl_3$), 12,13-*epoxydesmycosin* (**46**) (302 mg, 52%) (Found: C, 58.7; H, 7.9; N, 1.6. $C_{39}H_{65}NO_{15}$·0.1$CHCl_3$ requires C, 58.56; H, 8.19; N, 1.75%); *m/z* 788 (MH^+); $[\alpha]_D^{26}$ −33.5° ($CHCl_3$); $[\theta]_{240}$ −114.770, $[\theta]_{280}$ −3 430, $[\theta]_{330}$ −9 420 (MeOH); $\lambda_{max.}$(MeOH) 237 nm (14 910); $\nu_{max.}$($CDCl_3$) 3 515, 1 715, 1 690, 1 618, 1 313, 1 183, 1 163, and 1 058 cm^{-1}.

Method 2. The macrolide (1 mol equiv.) was dissolved in a mixture of acetonitrile (*x* ml) and water (*y* ml). DFA (*z* ml) was added and the mixture was stirred at 25 °C for 1 h. The solution was poured into a mixture of dichloromethane and water at pH 10—11 and worked up as usual.

(a) The macrolide (**51**) (250 mg) (*x* = 2.5, *y* = 20, *z* = 0.4) gave, after chromatography (30 × 2 cm; 4% MeOH in $CHCl_3$), 12,13-*epoxytylosin* (**52**) (75 mg, 31%) (Found: C, 56.8; H, 8.25; N, 1.3. $C_{46}H_{77}NO_{18}$·0.3$CHCl_3$ requires C, 57.09; H, 8.02; N, 1.45%); *m/z* 932 (MH^+); $[\alpha]_D^{26}$ −53.2° ($CHCl_3$); $[\theta]_{245}$

* Prepared by slowly adding titanium(III) chloride in small amounts, under argon, to stirred water at 0 °C.

$-78\,070$, $[\theta]_{275}$ 0, $[\theta]_{335}$ $-9\,460$ (MeOH); $\lambda_{max.}$(MeOH) 237 nm (13 120); $\nu_{max.}$($CDCl_3$) 3 675, 3 535, 1 718, 1 690, 1 615, 1 158, and 1 050 cm^{-1}, and 12,13-epoxydesmycosin (**46**) (121 mg, 61%).

(b) The macrolide (**53**) (178 mg) (x = 5.34, y = 11.5, z = 0.264) gave, after chromatography (30 × 2 cm; 1.75% MeOH in $CHCl_3$), *12,13-epoxy*-4″-O-*isovaleryltylosin* (**54**) (94 mg, 55%) (Found: C, 56.8; H, 8.25; N, 1.3. $C_{51}H_{85}NO_{19}$·0.5$CHCl_3$ requires C, 56.94; H, 7.97; N, 1.31%); m/z 1 016 (MH^+); $[\alpha]_D^{26}$ −53.2° ($CHCl_3$); $[\theta]_{245}$ −52 300, $[\theta]_{275}$ +7 850, $[\theta]_{330}$ −2 620 (MeOH); $\lambda_{max.}$(MeOH) 238 nm (13 230); $\nu_{max.}$($CDCl_3$) 3 660, 3 450, 1 725, 1 640, 1 620, 1 248, and 1 050 cm^{-1}.

3′-N-*Acetyl*-3′-N-*demethyl*-12,13-*epoxytylosin* 20-*Dimethyl Acetal* (**59**) (2:1 *Mixture of Rotamers*).—3′-*N*-Demethyl-12,13-epoxytylosin 20-dimethyl acetal (**58**) (20 mg) was dissolved in methanol (0.3 ml) and acetic anhydride (0.0098 ml) was added. The mixture was kept at 25 °C for 4.75 h. Chromatography (15 × 1.5 cm; 6% MeOH in $CHCl_3$) gave compound (**59**) (16.4 mg, 79%), $\nu_{max.}$($CDCl_3$) 3 520, 1 720, 1 690, 1 623, 1 167, 1 128, and 1 060 cm^{-1}.

2′,3,4‴-*Tri*-O-*acetyl*-12,13-*epoxy*-4″-O-*isovaleryltylosin* (**55**).—12,13-Epoxy-4″-*O*-isovaleryltylosin (**54**) (10.9 g), DMAP (2.6 g), and triethylamine (15 ml) were dissolved in dry dichloromethane (300 ml) and acetic anhydride (5.05 ml) was added. The mixture was kept at 25 °C for 18 h. Chromatography (30 × 5 cm; 15% Me_2CO in C_6H_{14}), gave 2′,3,4‴-*tri*-O-*acetyl*-12,13-*epoxy*-4″-O-*isovaleryltylosin* (**55**) (9.9 g, 81%) (Found: C, 59.55; H, 7.9; N, 1.2. $C_{57}H_{91}NO_{22}$ requires C, 59.93; H, 8.03; N, 1.22%); m/z 1 142 (MH^+); $[\alpha]_D^{26}$ −53.1° ($CHCl_3$); $[\theta]_{220}$ −63 460, $[\theta]_{280}$ +5 770 (MeOH); $\lambda_{max.}$(CF_3CH_2OH) 237 nm (14 280); $\nu_{max.}$($CDCl_3$) 3 500, 1 738, 1 690, 1 620, 1 238, 1 163, and 1 053 cm^{-1}.

2′,3,23-*Tri*-O-*acetyl*-23-O-*demycinosyl*-12,13-*epoxy*-4″-O-*isovaleryltylosin* (**61**).—3-*O*-Acetyl-23-*O*-demycinosyl-12,13-epoxy-4″-*O*-isovaleryltylosin (**60**) (519 mg), DMAP (14.3 mg), and pyridine (0.463 ml) were dissolved in dry dichloromethane (86.5 ml). Acetic anhydride (0.27 ml) was added and the mixture was kept at 25 °C for 22 h. Chromatography (30 × 2 cm; 16% Me_2CO in C_6H_{14}) gave 2′,3,23-*tri*-O-*acetyl*-23-O-*demycinosyl*-12,13-*epoxy*-4″-O-*isovaleryltylosin* (**61**) (502 mg, 88%) (Found: C, 59.45; H, 8.0; N, 1.35. $C_{49}H_{77}NO_{18}$·0.2$CHCl_3$ requires C, 59.33; H, 7.82; N, 1.41%); m/z 968 (MH^+); $[\alpha]_D^{26}$ −39.7° ($CHCl_3$); $[\theta]_{222}$ +71 340, $[\theta]_{248}$ −20 380, $[\theta]_{278}$ +7 640 (MeOH); $\lambda_{max.}$(MeOH) 239 (11 930) and 285 nm (2 080); $\nu_{max.}$($CDCl_3$) 3 490, 1 735, 1 690, 1 620, 1 233, 1 158, 1 048, and 1 020 cm^{-1}.

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