NMR Spectroscopic and X-Ray Crystallographic Studies on the Structure, Stereochemistry and Conformation of a Series of 9,11-Cyclic Aminals of (9*S*)-9-*N*-Methylerythromycylamine A

J. Sydney Davies,[a] Jeremy R. Everett,[a] Ian K. Hatton,*[,a] Eric Hunt,[a] John W. Tyler,[a] Iskander I. Zomaya,[a] Alexandra M. Z. Slawin[b] and David J. Williams[b]
[a] *Beecham Pharmaceuticals, Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ, UK*
[b] *Imperial College of Science and Technology, London SW7 2AZ, UK*

(9*S*)-9-*N*-Methylerythromycylamine A (**6**) and (9*S*)-9-*N*,*N*-dimethylerythromycylamine A (**7**) have been synthesised and their solution conformations compared with that of (9*S*)-erythromycylamine A (**5**) using ^{1}H and ^{13}C NMR spectroscopy. Compound **6** reacts with a number of aliphatic aldehydes to give 9,11-cyclic products which are shown to be diastereoisomeric about the bridging carbon atom C-23. Compounds with the same configuration at C-23 show close similarities in their ^{1}H and ^{13}C NMR spectra. In some cases, configurational interconversion at C-23 occurred by the well known 'ring-chain' tautomerism of tetrahydro-1,3-oxazines. The tetrahydro-1,3-oxazine rings of compounds with a (23*R*) configuration adopt a chair-like conformation while those compounds with a (23*S*) configuration prefer a twist-boat-like conformation. From NMR spectroscopic studies the solution conformations of the macrocyclic lactones of these compounds is best described as a fast equilibrium between C-3 to C-5 'folded out' and C-3 to C-5 'folded-in' types. Epimerisation at C-23 causes profound changes in the position of this equilibrium: compounds with the (23*S*) stereochemistry preferentially populate 'folded-out' conformations modelled on the crystal structure of erythromycin A hydroiodide dihydrate, while those possessing the (23*R*) configuration preferentially populate 'folded-in' conformational types similar to the crystal structure of (9*S*),(22*R*)-9,11-*N*,*O*-(2-methoxyethoxyethylidine)erythromycylamine A (**10**).

An example of each diastereoisomer at C-23 was chosen for X-ray diffraction studies. The crystal structures of (9*S*,23*S*)-9-*N*-methyl-9,11-*N*,*O*-(2-hydroxyethylidene)erythromycylamine A (**25**) and (9*S*,23*R*)-9-*N*-methyl-9,11-*N*,*O*-(3-ethoxypropylidene)erythromycylamine A (**28**) are thus reported and confirm the structural and conformational conclusions determined by NMR spectroscopy.

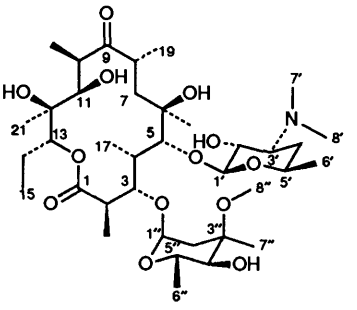
Erythromycin A † (**1**) is the most clinically important member of the macrolide class of orally active antibiotics despite its instability to mild acid.[1] Improved acid stability has been achieved by conversion of the ketone at C-9 into an oxime[2] **2** (or *O*-alkylated oxime[3] *e.g.* **3**) and into the (9*S*) amine[2b,4] **5** (termed erythromycylamine A) and numerous derivatives of these compounds have been synthesised in the search for improved chemotherapeutic agents. Several 11,12-cyclic derivatives of **1** have also been described, including the 11,12-cyclic carbonate,[5] the 11,12-cyclic sulphite,[6] 11,12-cyclic amide acetals,[7] and the 11,12-cyclic methylene acetal.[8] More recently, 9,11-cyclic acetal derivatives of (9*S*)-9-dihydroerythromycin A have been reported[9] with interesting antibacterial activity. Therefore as part of our programme of work aimed at synthesising novel erythromycin A analogues with improved pharmacokinetic and antibacterial properties, we decided to investigate the synthesis of cyclic derivatives of (9*S*)-erythromycylamine A (**5**).

(9*S*)-Erythromycylamine A (**5**) reacts[10,11] with aromatic aldehydes to give Schiffs' bases and with aliphatic ketones to give imines[10] both of which can be reduced with sodium borohydride giving *N*-benzylerythromycylamines and secondary amines, respectively. Aliphatic aldehydes (excluding formaldehyde) had been reported[10,11] to form cyclic aminals *e.g.* **10**[12] where the bridge was eventually proved[13] to be between the C-9 and C-11 positions of the macrocycle. Formaldehyde, however, gave a bis-adduct whose structure was assigned[11] as **14**. These aminals *e.g.* **10** were resistant to reduction[11] by sodium borohydride so the simple *N*-alkyl derivatives of (9*S*) erythromycylamine A **5** were not readily prepared.[10,11] No cyclic analogues of these *N*-alkyl erythromycylamines were known and so these compounds were chosen as our synthetic targets.

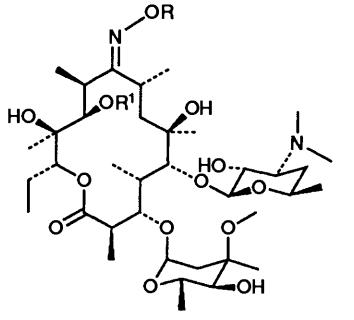
Synthesis of (9S)-9-N-*Methylerythromycylamine A* (**6**).—Formaldehyde reacted with (9*S*)-erythromycylamine A (**5**) in both ethanol and tetrahydrofuran (THF) solution to give the bis-adduct[11] **14** as the major product, whose ^{13}C NMR spectrum (Table 1) showed two methylene resonances at 82.5 and 77.9 ppm, corresponding to the 6,9-*O*,*N*- and 9,11-*N*,*O*-methylene bridges. Silica-gel chromatography of the bis-adduct **14** (eluting with chloroform–methanol–0.880 ammonia, 91:8:1), gave a little erythromycylamine A (**5**) and a pure product of identical R_f with the bis-adduct **14**. This compound was found to be the mono-adduct **11**. Only one bridging methylene resonance was now observed in the ^{13}C NMR spectrum (Table 1) at 75.7 ppm while C-6 had shifted to 74.3 ppm—compatible with it now possessing a free hydroxy group.

The 9,11-mono-adduct **11** was not reduced to **6** by sodium borohydride or lithium tri-t-butoxyaluminium hydride. However, the isolation of erythromycylamine A (**5**) from column chromatography of the mono-adduct **11** could be explained if silica catalysed the formation of the formaldehyde imine **15** which was subsequently hydrolysed to **5**. Thus when **11** was treated with was sodium cyanoborohydride in methanol solution acidified to pH 3–4 with 15% aqueous citric acid, the reduction proceeded smoothly to give consistently high yields

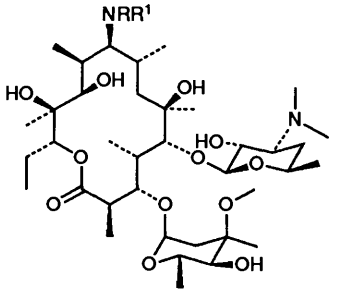
† The erythromycin A molecule consists of a polyfunctionalised 14-membered lactone ring termed erythronolide A, substituted by an amino sugar, D-desosamine and a neutral sugar, L-cladinose.



1



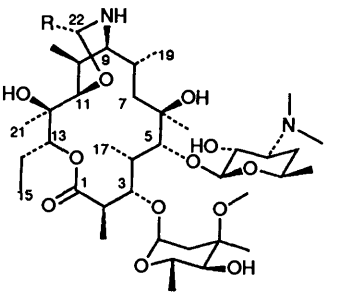
2 R = H, R^1 = H
3 R = CH_3, R^1 = H
4 R = CH_3, R^1 = $CH_2O(CH_2)_2N(CH_3)_2$



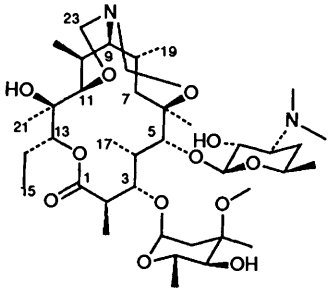
5 R = R^1 = H
6 R = H, R^1 = CH_3
7 R = R^1 = CH_3
8 R = H, R^1 = CH_3CH_2
9 R = H, R^1 = $HO(CH_2)_2$

(70–80%) of (9*S*)-9-*N*-methylerythromycylamine A (**6**). When the bis-adduct **14** was reduced under these conditions, (9*S*)-9-*N*,*N*-dimethylerythromycylamine A (**7**) was formed exclusively. To demonstrate the generality of these reaction conditions for this system, the 9,11-cyclic aminals formed by separate reaction of **5** with acetaldehyde and glycolaldehyde dimer *i.e.* **12** and **13**, respectively, were reduced and gave the secondary amines **8** and **9** in yields of 63 and 64% respectively.*

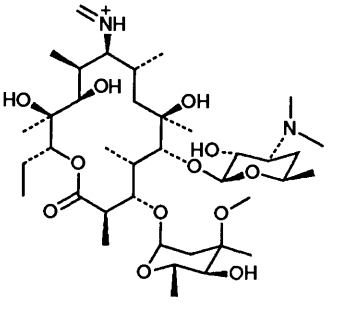
The Conformational Analysis of Erythromycins.—Extensive NMR work from these laboratories has shown [15–17] that the macrocyclic lactone rings of erythromycin A (**1**) and some of its C-9 derivatives, exist in $CDCl_3$ solution with their lactone rings



10 R = $CH_3O(CH_2)_2OCH_2$
11 R = H
12 R = $^{23}CH_3$
13 R = $HO^{23}CH_2$



14



15

in fast exchange between two types of conformations—the C-3 to C-5 'folded-out' type and the corresponding C-3 to C-5 'folded-in' type. The 'folded-out' type, conformation **A**, is based on the crystal structure of erythromycin A hydroiodide dihydrate,[22a] whilst the 'folded-in' conformations **B** and **B′** are based on the crystal structures of **10** [13] and **4**,[17] respectively (Fig. 1).

The 'folded-out' conformation **A** is characterised by a close cross-ring approach of 4-H and 11-H. In the 'folded-in' conformations **B** and **B′**, rotation, principally about the C-2 to C-3 and C-5 to C-6 bonds causes the inward folding of the C-3 to C-5 portion of the macrocyclic lactone ring. However, the relative orientation of the sugar rings with respect to one another remains approximately the same. When the C-3 to C-5 portion is folded-in, there is a close cross-ring approach of 11-H to 3-H. Thus, NOE experiments which probe the spatial proximities of 11-H to 3-H and 4-H provide an excellent monitor of the macrocyclic lactone ring folding about C-3 to C-5. Conformations **B** and **B′** differ from each other mainly in the C-6 to C-9 portions of the lactone ring.[17]

For (9*S*)-9-hydroxy-9-deoxoerythromycin A and (9*S*)-erythromycylamine A (**5**), the conformational equilibria were originally described [15] as involving conformations **A** and **B**, but with additional flexibility in the C-6 to C-9 region. Since the subsequent discovery [17] of the **B′** conformation of **4**, it is

* Since the completion of this work, **6** and **7** have been prepared [14] as a mixture by treating (9*S*)-erythromycylamine A **5** with 1 equiv. of 37% aqueous formaldehyde, pH 4.5 sodium phosphate buffer (0.5 mol dm^{-3}) and sodium cyanoborohydride in acetonitrile in a 'one-pot' procedure. Separation was only achieved [14] by reversed-phase preparative HPLC to give pure **6** and **7**, each in *ca.* 5% yield. Treatment of **5** with acetaldehyde under similar conditions [14] gave the *N*-ethyl product **8** in only 7% yield.

Table 1 ^{13}C and ^{1}H NMR chemical shifts for compounds **6–9**, **11** and **14** in $CDCl_3$–Me_4Si

Carbon or proton No.	δ_C(ppm)						δ_H(ppm)	
	14	**11**	**6**	**7**	**8**	**9**	**6**	**7**
1	174.9	176.9	177.7	177.1	177.4	177.4	—	—
2	44.5	44.5	44.8	44.5	44.7	44.8	2.90	2.69
3	80.5	77.1	79.8	78.5	79.1	79.3	4.19	3.88
4	37.5	44.3	39.7	44.3	40.6	40.2	1.99	1.75
5	83.0	79.4	83.8	83.7	83.6	83.7	3.60	3.62
6	79.2	74.3	76.3	74.5	75.9	76.0	—	—
7	36.5	39.0	36.3	39.5	37.1	36.9	1.48	1.68, 1.13
8	30.6	28.5	30.5	29.6	31.8	31.7	2.25	2.51
9	78.2	64.9	73.4	78.6	70.9	70.6	2.09	2.16
10	30.3	28.5	29.5	31.8	29.8	30.0	2.13	1.98
11	70.2	72.3	70.3	70.2	70.2	70.3	3.88	3.52
12	74.7	74.6	73.8	74.1	73.8	74.1	—	—
13	76.1	76.5	78.1	76.9	77.9	77.9	4.67	4.94
14	20.9	21.5	21.4	21.9	21.6	21.6	1.92, 1.53	1.97, 1.42
15	10.7	11.3	11.1	11.4	11.2	11.2	0.88	0.89
16	15.1	13.1	15.2	13.3	14.8	14.9	1.20	1.18
17	9.4	9.1	9.3	8.9	9.3	9.3	1.11	1.08
18	21.5	24.9	26.9	23.5	26.0	26.1	1.32	1.17
19	20.2	20.7	21.5	22.6	21.4	21.5	1.01	1.17
20	18.0	15.0	16.9	16.2	16.5	16.6	1.17	1.15
21	16.4	14.8	16.7	16.0	16.4	16.5	1.12	1.09
22	82.5	75.7	35.9	45.8	43.8	51.5	2.39	2.45
23	77.9				15.2	61.2		
1′	102.9	101.0	103.2	101.5	102.9	103.0	4.45	4.71
2′	71.3	71.1	71.0	70.9	70.9	71.0	3.26	3.30
3′	65.3	65.6	65.4	65.2	65.4	65.4	2.46	2.47
4′	29.1	28.8	28.8	28.8	28.8	28.8	1.67, 1.25	1.68, 1.25
5′	68.7	69.4	68.9	69.4	69.0	69.0	3.51	3.61
6′	21.5	21.1	21.4	21.3	21.3	21.3	1.23	1.24
7′ + 8′	40.3	40.4	40.3	40.4	40.3	40.3	2.29	2.31
1″	96.4	94.5	96.2	95.1	95.9	96.0	4.98	5.12
2″	35.3	34.5	35.0	34.6	34.9	35.0	2.38, 1.58	2.43, 1.60
3″	72.8	72.8	72.7	72.9	72.7	72.7	—	—
4″	78.0	78.3	78.0	77.8	77.9	77.9	3.04	3.03
5″	65.5	66.0	65.6	65.9	65.5	65.6	4.06	3.98
6″	18.7	18.3	18.4	17.8	18.4	18.4	1.33	1.25
7″	21.7	21.9	21.5	21.6	21.6	21.5	1.24	1.25
8″	49.5	49.2	49.4	49.3	49.4	49.4	3.32	3.37

possible that conformation **B′** is also involved in the conformational equilibria of these two compounds. However, for **4**, the conformational equilibria were found to be between conformations of type **A** and type **B′**, with no significant contributions from conformations like **B**.

The Conformational Analysis of Compounds **6** *and* **7**.—The ^{1}H and ^{13}C NMR spectra of **6** and **7** were unambiguously assigned (Table 1) using a variety of one-dimensional (1D) and two-dimensional (2D) NMR experiments, as previously described.[15,16,18]

For **6**, NOE difference spectroscopy experiments showed that the macrocyclic lactone ring was in fast exchange between conformations of type **A** (dominant) and type **B′** (minor) with no significant contributions from **B**-type conformations. Irradiation of 11-H produced an NOE at 4-H as well as 3-H (small), 8-H and both C-7 protons.

For **7**, irradiation of 11-H produced NOE's at 3-H, both C-7 protons and 8-H with virtually no NOE at 4-H. This indicated[15–18] that by contrast, the macrocyclic lactone ring of **7** was in fast exchange between conformations of type **B** and **B′** (both major) and type **A** (minor).

Reaction of (9S)-9-N-*Methylerythromycylamine A* (**6**) *with Aldehydes*.—Compound **6** did not react with aromatic aldehydes (*e.g.* benzaldehyde) or with aliphatic ketones (*e.g.* acetone), but did react very rapidly with 37% aqueous formaldehyde in ethanol* to give a single product, (9*S*)-9-*N*-methyl-9,11-*N*,*O*-methyleneerythromycylamine A (**16**). The ^{13}C NMR spectrum of **16** at room temperature had many signals broadened and some were absent. The spectrum was re-run at +50° and −50 °C and showed many signals shifted between the two temperatures (see Experimental).

N-Alkylerythromycylamines have been reported[14] to react poorly (if at all) with aldehydes other than formaldehyde. However, we report that **6** reacted with a number of aliphatic aldehydes (in ethanol or preferably THF) to give 9,11-cyclic products **21–28**. These products exist in two distinct forms which can interconvert in solution (*e.g.* chloroform at room temperature) (Type 1: less polar and Type 2: more polar product on silica-gel) (Table 2). The ratio and stability of the observed products were dependent on the aldehyde used and in some cases only one product was isolated. All members of the same type are characterised by close similarities in their ^{13}C and ^{1}H NMR spectra (Tables 3 and 4).

Structural Analysis of Compounds **23–28** *by* ^{1}H and ^{13}C NMR *Spectroscopy*.—The ^{13}C and ^{1}H NMR spectra of compounds **23–28** were unambiguously assigned (Tables 3 and 4) with the aid of various 1D and 2D NMR experiments in a manner similar to that described[15,16] previously. Analysis of the

* Subsequent to our experiments, the reactions of *N*-isopropyl-, *N*-benzyl-, *N*-cyclopentyl- and *N*-(3-dimethylamino-1-methyl)propylerythromycylamine A with formaldehyde have been reported[14] to give products **17–20**.

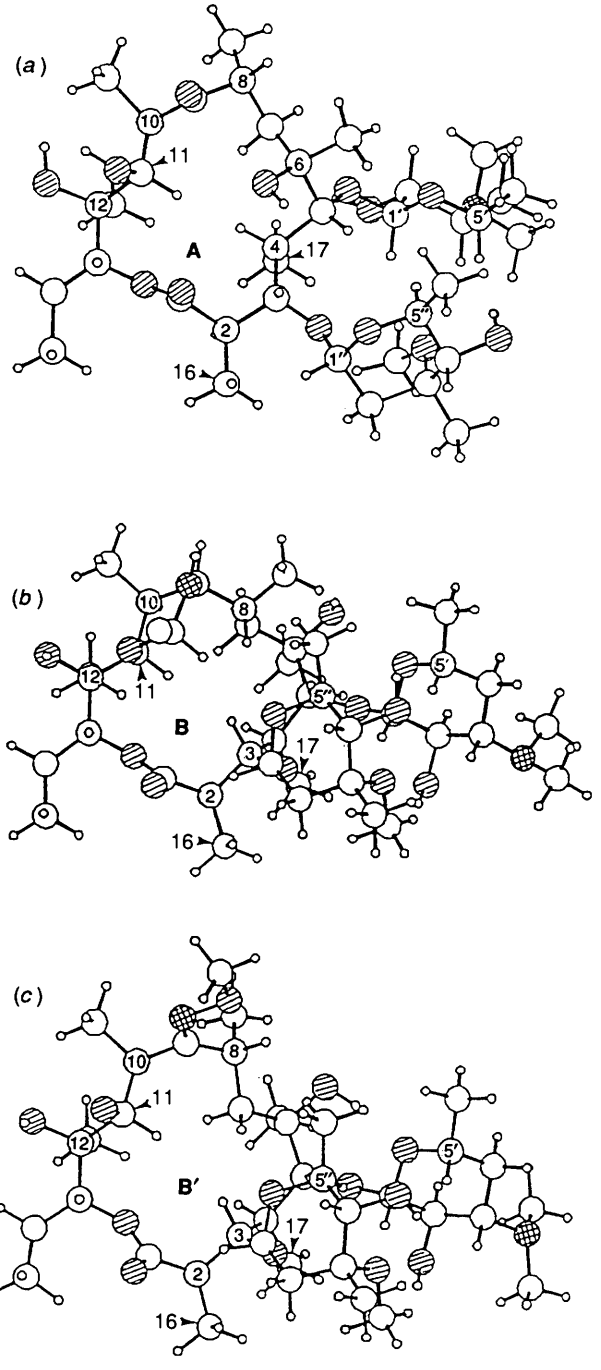
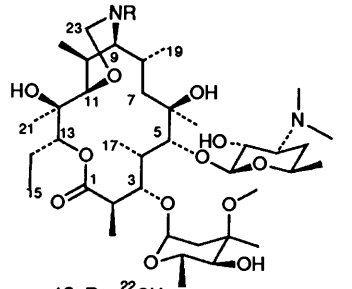
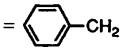
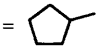
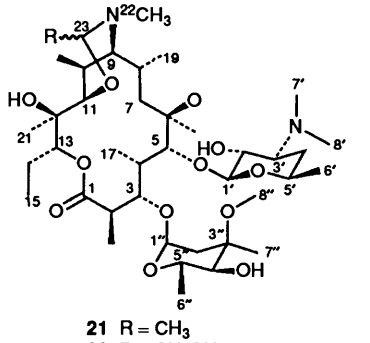


Fig. 1 The crystal structures of (*a*) erythromycin A hydroiodide dihydrate, (*b*) (9*S*),(22*R*)-9,11-*N*,*O*-(2-methoxyethoxyethylidene)erythromycylamine **A 10** and (*c*) *E*-11-*O*-(2-dimethylaminoethoxy)methyl-9-deoxo-9-methoxyiminoerythromycin A **4**. In both (*b*) and (*c*) the long ether side-chains have been omitted for clarity. Oxygen atoms are shaded and nitrogen atoms are cross-hatched.

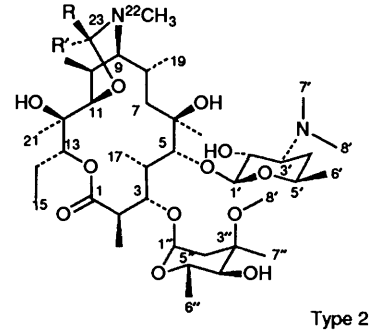
chemical shift and coupling constant data (Tables 3–5) showed that the compounds fell into two distinct groups; Type 1 (compounds **23**, **25** and **27**) and Type 2 (compounds **24**, **26** and **28**). The Type 2 compounds have NMR parameters very similar to those for known 9,11-cyclic aminals *e.g.* **10**. All members of the same type showed close similarities in their ^{1}H and ^{13}C NMR spectra. Some particularly characteristic differences in the ^{13}C chemical shifts between members of the two types can be seen for the erythronolide methine carbons C-5, C-7 and C-11, methyl groups C-16, C-18 and C-20 and the C-1′ carbon of the desosamine sugar. The same trends can be seen in a number of other parameters such as ^{1}H chemical shifts (Table 4) and macrolide vicinal coupling constants (Table 5).



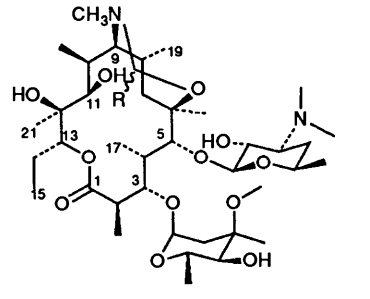
16 R = $^{22}CH_3$
17 R = $(CH_3)_2CH$
18 R = 
19 R = 
20 R = $(CH_3)_2N(CH_2)_2CH(CH_3)$



21 R = CH_3
22 R = CH_3CH_2



Type 1	Type 2
23 R = $PhCH_2$, R^1 = H	**24** R = H, R^1 = $PhCH_2$
25 R = $HO^{24}CH_2$, R^1 = H	**26** R = H, R^1 = Ph_2CH
27 R = $^{27}CH_3{}^{26}CH_2O(^{25}CH_2{}^{24}CH_2)$, R^1 = H	**28** R = H, R^1 = $C_2H_5O(CH_2)_2$



29 R = $PhCH_2$
30 R = $HOCH_2$

Several explanations were considered to account for the existence of the two types of product and their ability, in some cases, to interconvert in solution. One possible explanation was

Table 2 Experimental conditions for the preparation of compounds **21–28**

Compound **6** (wt/g)	Aldehyde (V/cm^3 or wt/g)	Solvent (V/cm^3)	Reaction time/h	Product (Type 1:Type 2) (wt/g)	Isolated separately
0.15	Acetaldehyde (0.5 cm^3)	EtOH (2)	0.5	**21** (1:5);[a] (0.15)	No
0.3	Propionaldehyde (1.0 cm^3)	EtOH (2)	16	**22** (1:5); [a] (0.25)	No
0.3	Phenylacetaldehyde (0.75 cm^3)	EtOH (4)	170	**23, 24** (1:1);[b] (0.085:0.082)	Yes
0.3	(0.75 cm^3)	THF (4)	16	**23, 24** (1:3);[b] (0.052:0.153)	Yes
2.0	Glycolaldehyde dimer (0.6 g)	THF (20)	48	**25** (8:1);[c] (1.82)	Yes
0.7	Diphenylacetaldehyde (1.5 g)	THF (8)	48	**26** (1:10);[c] (0.21)	Yes
0.2	3-Ethoxypropionaldhyde (0.5 cm^3)	THF (3)	2	**27, 28** (4:9);[b] (0.037; 0.087)	Yes[d]

[a] Ratio estimated by HPLC and ^{1}H NMR spectroscopy. [b] Isolated product ratio. [c] Ratio estimated by HPLC but only one isomer isolated. [d] Both products were isolated and characterised and then separately crystallised from ethyl acetate. The crystalline products were found to be identical and of Type 2. Thus **27** had isomerised and crystallised as **28**.

Table 3 ^{13}C NMR chemical shifts, δ_C(ppm) for Type 1 and Type 2 compounds **23–28** in $CDCl_3$–Me_4Si[a]

	Type 1 compounds			Type 2 compounds		
Carbon No.	**23**	**25**	**27**	**24**	**26**	**28**
1	175.0	176.0	175.0	175.9	176.0	176.9
2	44.9	45.1	44.8	44.0	44.7	44.5
3	80.2	80.3	79.9	79.6*	78.4*	76.6*
4	39.3	39.0	*ca.* 39*	44.3	44.5*	44.5*
5	84.7	84.3	84.6	81.5*	81.0*	79.2*
6	73.6[b]	73.6[b]	73.5	74.5	74.3	74.5
7	34.8	34.5	34.8	39.2	not observed	39.7*
8	29.7	29.3	29.6	29.9	29.4	30.2
9	73.8	70.6	73.9	77.1	76.7	75.5*
10	29.2	29.3	29.3	29.1	29.0	29.0
11	69.6	70.6	69.7	74.3	74.7	74.4
12	74.1[b]	73.9[b]	74.1	74.5	75.0	74.5
13	76.1	76.4	75.6	77.0	77.4	76.4
14	21.5	21.0	21.5	21.1	21.6	21.3
15	11.1	10.5	10.8	11.7	11.4	11.3
16	16.2	16.2	15.9	12.9	13.3	13.1
17	9.4	9.3	9.3	9.3	9.4	9.0
18	27.6	27.6	27.2*	24.8	*ca.* 26*	24.7
19	21.1	21.2	20.9*	20.8	19.8	21.2
20	20.0	20.2	20.1	16.3	16.2	16.4
21	15.3	15.3	15.4	14.9	15.0	14.8
22	38.0	36.6	37.5	41.4*	40.5	40.4
23	91.0	90.0	87.8	89.0	89.9	86.9
24	41.4	62.8	34.5	39.9	54.7	33.3
25			65.4			68.0
26			66.0			66.0
27			15.3			15.3
1′	103.2	103.5	103.1	101.0	101.2	100.9
2′	71.2	71.0	71.1	71.1	71.1	71.0
3′	65.4	65.4	65.5	65.0	65.1	64.9
4′	29.0	28.8	28.9	29.0	28.9	28.9
5′	68.7	68.8	68.8	69.3	69.3	69.4
6′	21.5	21.4	21.5	21.2	21.3	21.1
7′ + 8′	40.3	40.3	40.3	40.4	40.4	40.4
1″	96.2	96.4	96.0	94.5	95.7	94.2
2″	35.2	35.0	35.1	34.7	35.0	34.5
3″	72.7	72.6	72.7	72.7	72.8	72.7
4″	78.1	77.8	78.1	78.7	78.2	78.6
5″	65.5	65.4	65.4	66.0	66.2	65.9
6″	18.6	18.5	18.5	18.5	18.8	18.4
7″	21.6	21.5	21.6	21.9	21.7	21.9
8″	49.5	49.4	49.5	49.3	49.3	49.2

[a] An asterisk refers to very broad resonances. [b] Assignments may be interchanged.

that both 6,9- and 9,11-cyclic aminals were present *e.g.* **23** or **24** and **29**. Type 2 compounds gave spectra very similar to known 9,11-cyclic aminals *e.g.* **10** and thus Type 1 compounds would probably have the 6,9-bridge in this case. Analysis of the ^{13}C NMR spectra of the Type 1 and Type 2 members did not support this hypothesis since the chemical shifts for C-5, C-6 and C-7 did not undergo the changes expected for alkylation of the 6-hydroxy group. Proof that C-6 bore an hydroxy group in Type 1 compounds was provided by a deuterium isotope effect experiment (SIMPLE NMR)[19] conducted upon **25**. The resonance of C-6 exhibited a significant isotope effect ($^2\Delta \sim -80$ ppb) on partial deuteriation of the hydroxy groups in the sample whilst C-11 did not, showing that C-6 and not C-11 possessed a hydroxy group. The structure of **25** (Type 1) was thus also confirmed as a 9,11-cyclic aminal and not its 6,9-isomer **30**.

Table 4 ^{1}H NMR chemical shifts, δ_H(ppm) for Type 1 and Type 2 compounds **23–28** in $CDCl_3$–Me_4Si[a]

	Type 1 compounds			Type 2 compounds		
Carbon No.	**23**	**25**	**27**	**24**	**26**	**28**
1	—	—	—	—	—	—
2	2.90	2.86	2.86	2.57	2.60	2.70
3	4.26	4.17	4.18	*ca.* 3.90	4.00	4.01
4	2.09	1.89	*ca.* 2.05	*ca.* 1.70	*ca.* 1.7	1.71
5	3.62	3.53	3.57	*ca.* 3.90	3.85	3.92
6	—	—	—	—	—	—
7	1.60, 1.50	1.60, 1.50	1.57, 1.45	not observed	1.45, 1.35	1.45, 1.35
8	2.41	2.40	2.42	2.18	2.41	2.09
9	1.74	1.72	1.71	*ca.* 3.90	2.18	2.14
10	1.97	1.98	1.98	1.94	1.99	1.91
11	3.86	3.72	3.81	3.17	3.13	3.13
12	—	—	—	—	—	—
13	5.15	5.07	5.11	4.29*	3.85*	4.92
14	1.78, 1.24	1.84, 1.50	1.88, 1.45	1.81, 1.24	1.68, 1.20	1.95, 1.45
15	0.85	0.78	0.83	0.78	0.66	0.89
16	1.23	1.13	1.18	1.11	1.18	1.19
17	1.09	1.03	1.08	1.06	1.11	1.09
18	1.36	1.31	1.31	1.12	1.14	1.11
19	0.98	0.93	0.97	1.29	1.35	1.26
20	0.99	1.06	1.10	1.23	1.25	1.23
21	1.04	1.04	1.11	1.03	1.02	1.06
22	2.33	2.24	2.22	2.30	2.52	2.65
23	4.12	3.91	4.16	4.83	5.26	4.57
24	3.08, 2.88	3.71, 3.67	2.01, 1.89	2.97, 2.83	4.19	1.95, 1.83
25			3.73, 3.62			3.63, 3.48
26			3.56, 3.48			3.53, 3.51
27			1.19			1.22
1′	4.48	4.34	4.46	4.77	4.84	4.80
2′	3.36	3.19	3.25	3.35	3.32	3.31
3′	2.53	2.39	2.42	2.50	2.52	2.53
4′	1.60, 1.22	1.61, 1.22	1.63, 1.23	1.65, 1.22	1.71, 1.22	1.65, 1.23
5′	3.51	3.42	3.49	3.63	3.72	3.62
6′	1.24	1.17	1.23	1.24	1.25	1.21
7′ + 8′	2.23	2.23	2.30	2.30	2.23	2.30
1″	4.98	4.87	4.95	4.91	5.27	5.30
2″	2.35, 1.62	2.28, 1.50	2.38, 1.56	2.42, 1.55	2.50, 1.62	2.45, 1.56
3″	—	—	—	—	—	—
4″	3.05	2.95	3.01	3.02	3.11	3.02
5″	4.06	3.99	4.04	*ca.* 3.90	4.12	3.88
6″	1.33	1.27	1.32	1.31	1.53	1.21
7″	1.25	1.17	1.24	1.28	1.29	1.28
8″	3.32	3.24	3.32	3.32	3.38	3.39

[a] An asterisk signifies shielding by aromatic ring currents.

Table 5 Vicinal ^{1}H,^{1}H coupling constants for Type 1 and Type 2 compounds **23–28** in $CDCl_3$–Me_4Si[a]

Spin system proton No.	Type 1 vicinal ^{1}H,^{1}H couplings (^3J/Hz)			Type 2 vicinal ^{1}H,^{1}H couplings (^3J/Hz)		
	23	**25**	**27**	**24**	**26**	**28**
16,2	7.0	7.0	7.2	7.1	7.2	7.1
2,3	9.8	10.3	9.0	*ca.* 2.0	2.0	2.0
3,4	1.1	<0.5	1.2	obs.	obs.	2.1
4,5	7.6	8.1	7.4	obs.	*ca.* 3.5	4.2
4,17	7.5	7.4	7.4	7.1	6.0	7.3
8,9	1.2	*ca.* 1.0	1.3	obs.	9.8	9.2
8,19	7.0	7.0	7.0	7.0	6.1	6.0
9,10	2.3	*ca.* 1.0	2.6	*ca.* 1.0	*ca.* 1.0	<0.5
10,11	2.2	2.3	2.2	*ca.* 1.0	*ca.* 1.0	*ca.* 1.0
10,20	7.1	7.2	7.3	7.1	7.2	7.0
13,14A	2.4	2.1	2.4	2.9	2.5	2.5
13,14B	10.7	11.3	10.8	8.5	9.9	10.2
14,15	7.3	7.2	7.3	7.5	7.4	7.4
23-24A	2.4	small	2.6	9.0		3.0
23-24B	9.2	small	*ca.* 6.7	3.1	*ca.* 9.6	8.9

[a] $^3J_{7,8}$ values observed, obs. = obscured. A, B denote individual methylene protons.

NOE Results and Discussion.—NOE experiments[15,16] were performed on compounds **23–25**, **27** and **28** and again a clear division of results was found between Type 1 and 2 compounds, but a strong similarity in results existed amongst members of the same group.

The most important results were those obtained for irradiation/observation of 9-H and 11-H (Fig. 2). The C-11 stereochemistry is fixed and the stereochemistry at C-9 was confirmed as *S* by the following NOE results. If the stereochemistry at C-9 was *R*, 9-H and 11-H would both be axial and on the same face of the tetrahydro-1,3-oxazine ring giving an expected moderate NOE between them. If C-9 had the *S*-configuration, 9-H is on the β-face of the macrolide while 11-H is α-orientated and any NOE between them would be expected to be very weak. Since members of both types showed only weak NOEs between 9-H and 11-H, this is interpreted as proving that the 9*S* stereochemistry is intact. Type 1 members exhibited significant NOEs between 9-H and 23-H while no NOEs were observed between 11-H and 23-H. Type 2 members, however, showed strong NOEs between 11-H and 23-H while interactions between 9-H and 23-H were very weak or absent.

The only interpretation consistent with all the NMR data invoked both a configurational inversion at C-23 and a

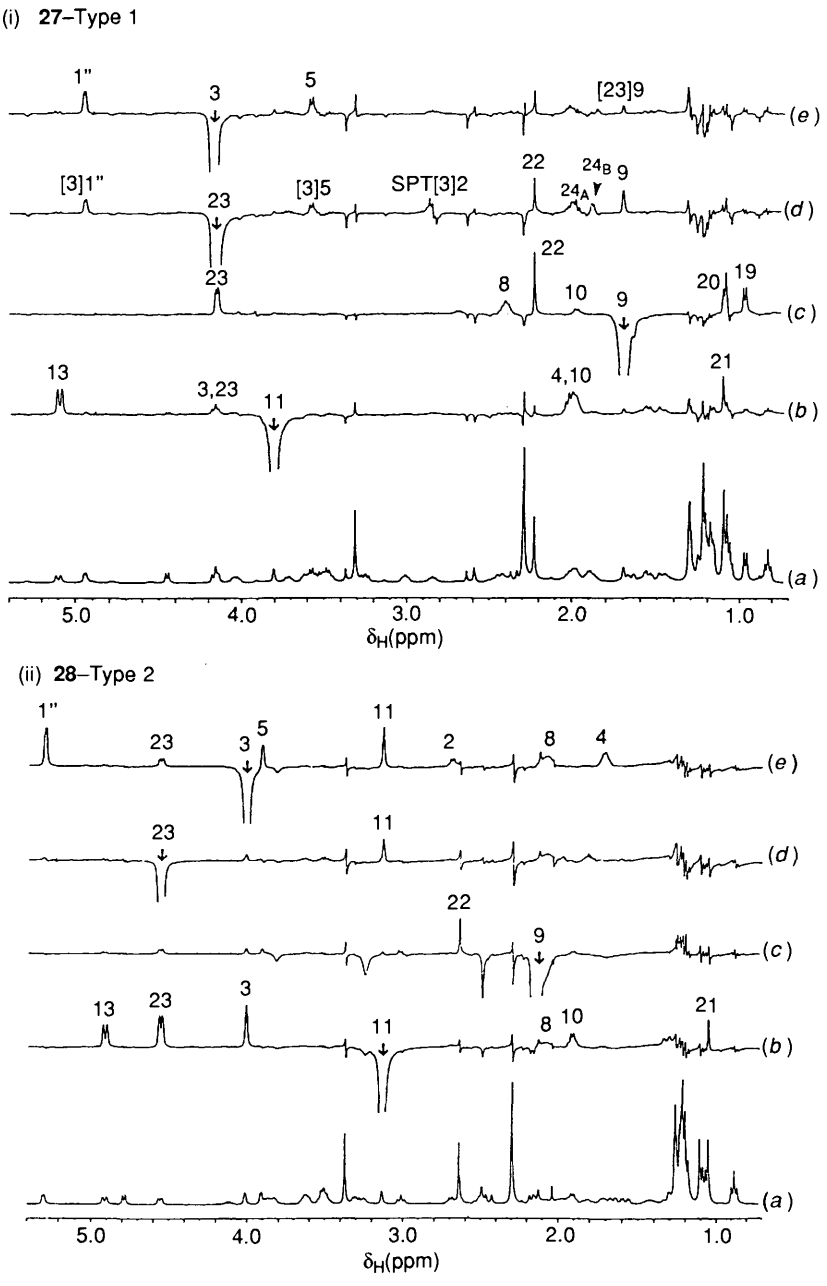


Fig. 2 400 MHz ^{1}H and ^{1}H NOE difference spectra produced by irradiation of (*b*) 11-H, (*c*) 9-H, (*d*) 23-H and (*e*) 3-H for (i) Type 1 compound **27** with 23*S* stereochemistry and (ii) Type 2 compound **28**, the 23*R* isomer of **27**

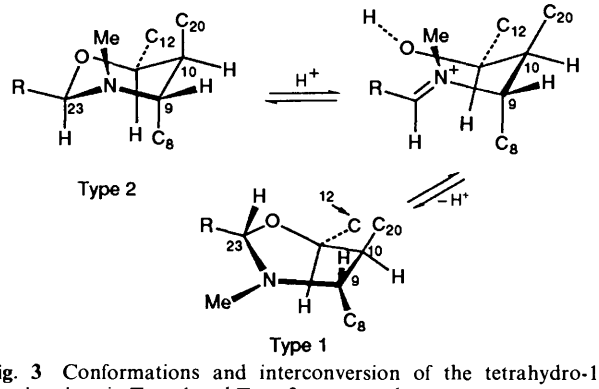


Fig. 3 Conformations and interconversion of the tetrahydro-1,3-oxazine rings in Type 1 and Type 2 compounds

conformational change in the tetrahydro-1,3-oxazine ring, between the Type 1 and Type 2 compounds. The Type 1 compounds have 23*S* stereochemistry and the tetrahydro-1,3-oxazine ring in a twist-boat-like conformation, whilst Type 2 compounds have 23*R* stereochemistry and a chair conformation for the tetrahydro-1,3-oxazine ring (Fig. 3).

Extensive work has been published[20] on tetrahydro-1,3-oxazines and their ability to undergo 'ring-chain' tautomerism *via* a planar intermediate (Fig. 3). This process provides a rationale for the inversion of configuration at C-23 in these compounds, *e.g.* **23** and **24**; and **27** and **28** (Table 2).

This epimerisation about C-23 also caused profound changes in the conformations of the macrolide rings. Complete analysis of the NOE data sets (Table 6) for compounds **23**–**28** (excluding **26**, which was not done) in a manner similar to that already described[17] shows that while these compounds exist as a conformational blend with rapid exchange between conformational types, there was a division into two groups. Those designated as Type 1 were all found preferentially to populate a conformation modelled on **A**[15] (Fig. 1) with its characteristic 'folded-out' C-3 to C-5 fragment. Type 2 members preferentially populated a conformation modelled on **B**[15] with C-3 to C-5 'folded-in' (Fig. 1).

Table 6 NOEs observed for experiments on compounds **23–25**, **27** and **28**[a]

Compound	Proton irradiated	NOEs observed
23	9-H	8-H (m), 10-H (s), 11-H (s), 19-Me (m), 20-Me (m), 22-Me (m), 23-H (m)
Type 1	11-H	3-H (s), 4-H (m), 9-H (s), 10-H (m), 13-H (m), 21-Me (m)
	13-H	11-H (m), 14-H (m), 15-Me (m), Ar (m)
	23-H	9-H (m), 11-H (s), 19-Me (m), 20-Me (s)
	1′-H	5-H (m), 17-Me (m), 3′-H (m), 5′-H (m), 5″-H (m)
	1″-H	3-H (l), 16-Me (s), 2″ax-H (m), 2″eq-H (s)
24[b]	11-H	3-H (m), 10-H (m), 13-H (m), 21-Me (m), 23-H (m)
Type 2	13-H	11-H (m), 14-H (m), 15-H (m), Ar (m)
	23-H	3-H (s), 9-H (s), 11-H (m), Ar (s)
	1′-H	5-H (m), 17-Me (s), 3′-H (m), 5′H (m), 5″-H (m), 8″-Me (m)
	1″-H	3-H (l), 16-Me (m), 2″ax-H (m), 2″eq-H (s)
25[c]	3-H	2-H (s), 4-H (s), 5-H (m), 1″-H (m)
Type 1	5-H	3-H (m), 18-Me (m), 1′-H (m), 5″-H (m)
	10-H	9-H (s), 11-H (m), 19-Me (m), 20-Me (m), 21-Me (s)
	11-H	3-H (s), 4-H (m), 7-H (m), 9-H (s), 10-H (s), 13-H (m), 21-Me (s)
	23-H	9-H (m), 11-H (s), 19-Me (s), 20-Me (s), 22-Me (m)
27	3-H	2-H (s), 4-H (s), 5-H (m), 11-H (s), 1″-H (m)
Type 1	9-H	8-H(m), 10-H(s), 11-H(s), 19-Me(m), 20-Me(m), 21-Me(s), 22-Me(m), 23-H(m)
	11-H	3-H (s), 4-H (m), 9-H (s), 10-H (m), 13-H (m), 21-Me (m), 23-H (s)
	13-H	11-H (m), 14-H (s), 15-Me (m)
	23-H	9-H (m), 11-H (s), 22-Me (s), 24-H_A (m), 24-H_B (m)
	1′-H	5-H (l), 3′-H (m), 5′-H (m), 5″-H (m)
	1″-H	3-H (l), 16-Me (s), 2″ax-H (m), 2″eq-H (s)
28	3-H	2-H (s), 4-H (m), 5-H (m), 8-H (m), 11-H (m), 23-H (s), 1″-H (m)
Type 2	9-H	3-H (s), 10-H (s), 11-H (s), 22-Me (m), 23-H (s)
	11-H	3-H (m), 8-H (s), 10-H (s), 13-H (m), 21-Me (s), 23-H (m)
	13-H	11-H (m), 14-H (s), 15-Me (m)
	23-H	3-H (s), 8-H (s), 9-H (s), 11-H (m)
	1′-H	5-H (m), 17-Me (s), 3′-H (m), 5′-H (m), 5″-H (m), 8″-Me (s)
	1″-H	3-H (l), 16-Me (m), 2″ax-H (m), 2″eq-H (s)

[a] l = large, > 5%; m = medium, > 1%, < 5%; s = small, < 1%. [b] 9-H coincident with 3-H, 5-H and 5″-H. [c] 11-H overlaps both 24-Hs.

In chloroform solution at room temperature, interconversion between Types 1 and 2, for most diastereoisomeric pairs (except **21** and **22**), was slow and depending on the substituent at C-23, could take hours or days. In the cases of **25** and **26** no epimerisation at C-23 was observable. This slow interconversion allowed separation of some Type 1 and Type 2 members with the same substituent at C-23.

The ratio of Type 1 and/or Type 2 products isolated from these reactions (Table 2) is also dependent on the nature of the substituent at C-23. The reactions were monitored by TLC and HPLC and in all cases, the Type 2 product was formed initially with Type 1 products being formed as the reaction progressed further (except for **26** when no Type 1 product was observed).

X-Ray Crystal Structures of **25** *Type* 1 *and* **28** *Type* 2 *Compounds*.—The crystal structures of **25** (Type 1) and **28** (Type 2) are shown in Figs. 4 and 5 respectively. Fractional atomic coordinates for the non-hydrogen atoms are given in Tables 7 and 10, bond lengths in Tables 8 and 11 and bond angles in Tables 9 and 12.

The stereochemistry and conformations of the tetrahydro-1,3-oxazine rings in **25** and **28** are different. For **25** (Type 1), the stereochemistry at C-23 is *S* while for **28** (Type 2), it is *R*, in agreement with the predictions of the earlier NMR experiments. In Table 13 the torsion angles around the tetrahydro-1,3-oxazine rings in both **25** and **28** are compared with those for the 9,11-cyclic erythromycylamine derivative **10**. Literature values[21] for the chair, boat and twist-boat conformations of cyclohexane are presented in Table 14. The tetrahydro-1,3-oxazine ring in **25** adopts a conformation which approximates to the cyclohexane twist-boat in which the C-23 substituent, C-8 and C-12 occupy quasi-equatorial positions and C-20 is in a quasi-axial conformation. In **28** and **10**, the tetrahydro-1,3-oxazine ring adopts a conformation which approximates to the cyclohexane chair, which again ensures that the C-23 substituent occupies an equatorial position; C-12 is also equatorial while C-8 and C-20 are axial. These results confirm the conclusions reached from the NMR experiments.

In Table 15, the torsion angles around the macrocyclic lactone are compared for erythromycin A (**1**), the erythromycin A-9-methoxime derivative **4**[17] and the erythromycylamine derivatives **10**,[13] **25** and **28**. The crystal structures of compounds **1**, **10** and **4** are the models for the conformations **A**, **B** and **B′** respectively (Fig. 1). In agreement with the results of the NMR experiments described earlier, the X-ray data shows that **25**-Type 1 adopts a C-3 to C-5 'folded-out' conformation similar to **A**, while **28**-Type 2 adopts a C-3 to C-5 'folded-in' conformation similar to **B**[15] in the crystalline state (Fig. 6). In agreement with the crystal structures of other derivatives,[13,22] both sugar rings in **25** and **28** adopt the chair conformation and are orientated with respect to each other as in erythromycin A.[22a]

Thus it is clear from the above results that the configurational change at C-23 drives conformational changes not only in the tetrahydro-1,3-oxazine ring but also in the macrocyclic lactone.

Conclusions

9-*N*-Methylerythromycylamine A (**6**) was prepared in good yield and was treated with a number of aldehydes to give 9,11-cyclic products which were diastereoisomeric about the nitrogen–oxygen bridging carbon atom. The ratio and stability of these diastereoisomers was dependent upon the aldehyde chosen. Epimerisation at C-23 was shown to result in

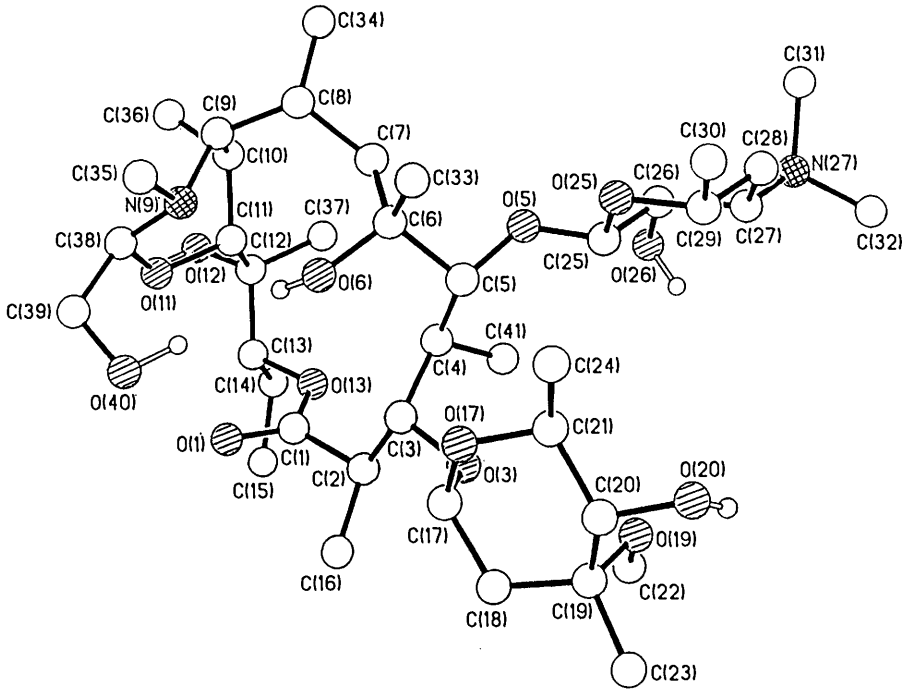


Fig. 4 The crystal structure of the Type 1 compound **25** showing the crystallographic numbering scheme and the macrocycle in conformation **A**. Intramolecular hydrogen bonds: O(6)···N(9) = 2.71, H···N = 2.07 Å, O–H···N 121°; O(20)···O(19) = 2.67, H···O = 2.26 Å, O–H···O 104°. There are intermolecular hydrogen bonds: O(12)···O(20′) = 2.78, H···O = 1.82 Å, O–H···O 167°; O(20)···O(11′) = 2.90, H···O = 2.21 Å, O–H···O 126°.

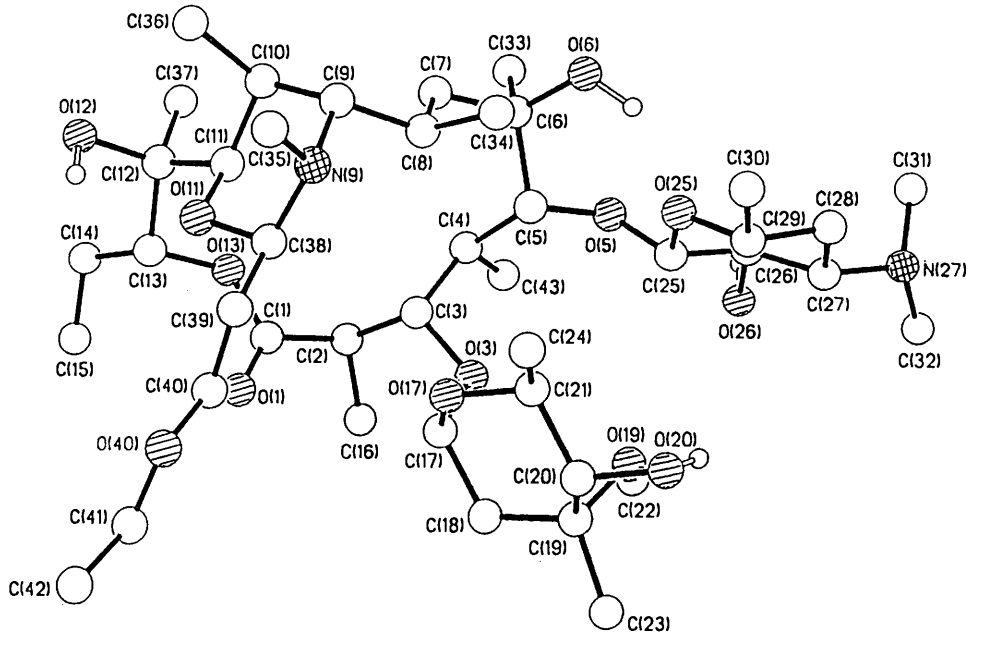


Fig. 5 The crystal structure of the Type 2 compound **28** showing the crystallographic numbering scheme and the macrocycle in conformation **B**. Intramolecular hydrogen bonds: O(6)···O(25) = 2.74, H···O = 1.85 Å, O–H···O 149°; O(12)···O(11) = 2.77, H···O = 2.18 Å, O–H···O 117°; O(20)···O(19) = 2.67, H···O = 2.10 Å, O–H···O 115°. Intermolecular hydrogen bonds: O(12)···O(6′) = 2.83, H···O = 2.25 Å, O–H···O 117°; O(26)···N(27′) = 2.95, H···N = 2.05 Å, O–H···N 152°.

conformational changes in both the tetrahydro-1,3-oxazine ring and the macrocyclic lactone ring of the compounds investigated. The antibacterial activities of compounds **23**–**28** were compared with those of erythromycin A (**1**) *in vitro*. Compound **25** showed equivalent activity to that observed for **1** against sensitive Gram-negative organisms and staphylococci but was less active than **1** against certain strains of streptococci. The other compounds were generally less active than **25** with no significant differences being observed between diastereoisomeric pairs (*e.g.* **23** and **24**; **27** and **28**), probably because of the ease of epimerisation of these compounds under the biological testing procedure.

Despite its interesting activity *in vitro*, **25** showed no advantage over **1** in treating experimental infections in mice and does not warrant further evaluation.

Experimental

The 1D ^{1}H and ^{13}C NMR experiments and the 2D ^{1}H COSY-45, 2D ^{1}H, ^{13}C COSY and 2D ^{1}H, ^{13}C COLOC experiments were performed as described previously[15,16,18] on a Bruker AM400 NMR spectrometer using a 5 mm ^{1}H/^{13}C dual probe at ambient temperature and sample concentrations of 30–60 mg

Table 7 Atom coordinates ($\times 10^4$) for **25** with estimated standard deviations (esds) in parentheses

Atom	*x*	*y*	*z*
O(1)	7248(2)	2111(2)	3272(2)
C(1)	6923(3)	1989(3)	2869(2)
C(2)	7190(3)	1698(3)	2342(2)
C(3)	7384(3)	2210(2)	1911(2)
O(3)	7719(2)	1910(2)	1456(1)
C(4)	6814(3)	2576(3)	1680(2)
C(5)	7032(3)	3160(3)	1337(2)
O(5)	6538(2)	3345(2)	947(1)
C(6)	7171(3)	3761(3)	1705(2)
O(6)	7546(2)	3549(2)	2171(2)
C(7)	6552(3)	4066(3)	1895(2)
C(8)	6588(3)	4632(3)	2300(3)
C(9)	6578(3)	4468(3)	2940(3)
N(9)	7189(2)	4152(2)	3123(2)
C(10)	5986(3)	4081(3)	3110(3)
C(11)	6193(3)	3406(3)	3204(2)
O(11)	6629(2)	3373(2)	3673(2)
C(12)	5659(3)	2915(3)	3309(2)
O(12)	5319(2)	3048(2)	3819(2)
C(13)	5947(3)	2248(3)	3376(2)
O(13)	6283(2)	2065(2)	2859(1)
C(14)	5438(4)	1728(3)	3464(3)
C(15)	5724(5)	1106(4)	3629(4)
C(16)	7741(4)	1277(3)	2509(3)
C(17)	8397(3)	1975(3)	1453(2)
O(17)	8601(2)	2541(2)	1198(2)
C(18)	8698(3)	1406(3)	1176(2)
C(19)	8684(3)	1385(3)	541(3)
O(19)	8037(2)	1360(2)	312(2)
C(20)	8916(3)	2025(3)	329(2)
O(20)	8870(2)	2069(2)	−263(2)
C(21)	8556(3)	2572(3)	600(2)
C(22)	7639(4)	861(4)	485(4)
C(23)	9090(4)	864(4)	311(3)
C(24)	8837(4)	3204(4)	434(3)
C(25)	6656(3)	3201(3)	383(2)
O(25)	7062(2)	3684(2)	163(1)
C(26)	6004(3)	3200(3)	76(2)
O(26)	5622(2)	2685(2)	274(2)
C(27)	6110(3)	3117(4)	−551(3)
C(28)	6612(3)	3601(4)	−766(3)
C(29)	7218(3)	3583(4)	−421(2)
C(30)	7667(4)	4099(5)	−578(3)
N(27)	5479(3)	3117(4)	−850(2)
C(31)	5233(4)	3776(6)	−964(4)
C(32)	5515(5)	2708(7)	−1347(4)
C(33)	7575(3)	4218(3)	1367(3)
C(34)	6034(3)	5094(3)	2178(3)
C(35)	7734(3)	4590(3)	3140(3)
C(36)	5633(3)	4391(3)	3606(3)
C(37)	5167(3)	2950(3)	2842(2)
C(38)	7108(3)	3853(3)	3678(2)
C(39)	7706(3)	3565(4)	3916(3)
O(40)	8007(3)	3140(4)	3558(3)
C(41)	6373(3)	2146(3)	1349(3)
O(51)	4537(5)	848(5)	2065(4)
O(52)	5867(5)	475(4)	2060(4)
O(53)	5977(8)	1397(4)	58(5)
O(54)	8857(4)	3308(5)	2295(7)
O(55)	4471(6)	780(4)	875(4)
O(56)	5914(8)	480(5)	887(5)

Table 8 Bond lengths/Å for **25** with esds in parentheses

O(1)–C(1)	1.204(7)	C(1)–C(2)	1.507(7)
C(1)–O(13)	1.337(7)	C(2)–C(3)	1.548(7)
C(2)–C(16)	1.503(9)	C(3)–O(3)	1.439(6)
C(3)–C(4)	1.517(8)	O(3)–C(17)	1.412(7)
C(4)–C(5)	1.550(7)	C(4)–C(41)	1.512(8)
C(5)–O(5)	1.441(6)	C(5)–C(6)	1.571(7)
O(5)–C(25)	1.402(6)	C(6)–O(6)	1.431(6)
C(6)–C(7)	1.507(8)	C(6)–C(33)	1.514(9)
C(7)–C(8)	1.540(8)	C(8)–C(9)	1.567(9)
C(8)–C(34)	1.538(9)	C(9)–N(9)	1.497(8)
C(9)–C(10)	1.530(8)	N(9)–C(35)	1.462(8)
N(9)–C(38)	1.479(7)	C(10)–C(11)	1.508(8)
C(10)–C(36)	1.539(9)	C(11)–O(11)	1.442(6)
C(11)–C(12)	1.539(8)	O(11)–C(38)	1.421(7)
C(12)–O(12)	1.435(7)	C(12)–C(13)	1.541(8)
C(12)–C(37)	1.513(8)	C(13)–O(13)	1.469(7)
C(13)–C(14)	1.541(9)	C(14)–C(15)	1.495(12)
C(17)–O(17)	1.408(7)	C(17)–C(18)	1.509(9)
O(17)–C(21)	1.433(7)	C(18)–C(19)	1.518(8)
C(19)–O(19)	1.450(8)	C(19)–C(20)	1.524(9)
C(19)–C(23)	1.492(11)	O(19)–C(22)	1.402(10)
C(20)–O(20)	1.421(7)	C(20)–C(21)	1.522(9)
C(21)–C(24)	1.511(10)	C(25)–O(25)	1.425(7)
C(25)–C(26)	1.538(8)	O(25)–C(29)	1.448(7)
C(26)–O(26)	1.428(8)	C(26)–C(27)	1.522(8)
C(27)–C(28)	1.550(11)	C(27)–N(27)	1.491(9)
C(28)–C(29)	1.503(9)	C(29)–C(30)	1.483(12)
N(27)–C(31)	1.511(15)	N(27)–C(32)	1.472(14)
C(38)–C(39)	1.495(9)	C(39)–O(40)	1.389(10)

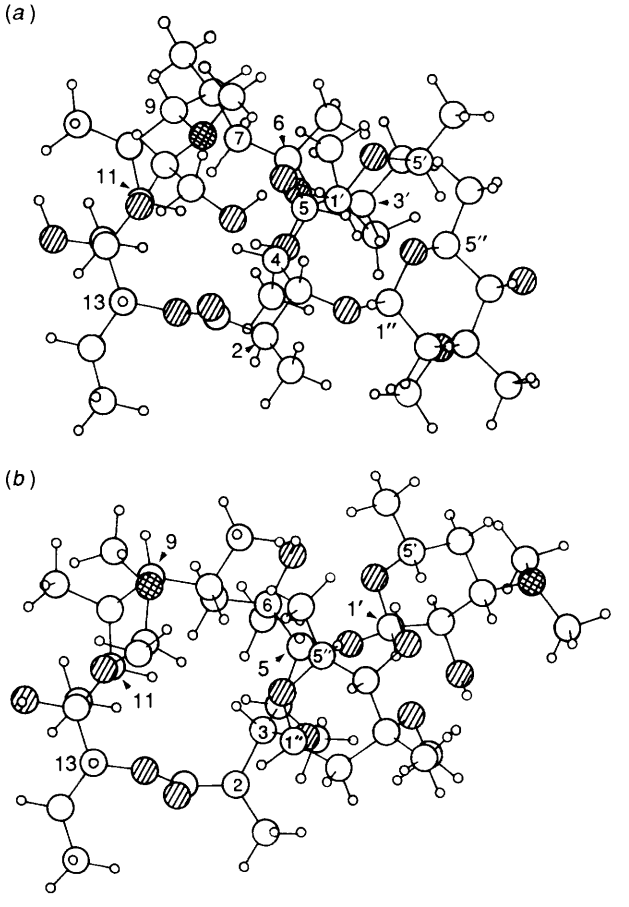


Fig. 6 The crystal structures of (*a*) **25** and (*b*) **28** viewed along the 13-H to C-13 bond. Oxygen atoms are shaded, nitrogen atoms are cross-hatched and the side-chain unit $C_2H_5OCH_2$ for **28** has been omitted for clarity.

cm^{-3} in $CDCl_3$. Fast atom bombardment mass spectra (FAB-MS) were obtained using a VG ZAB IF mass spectrometer operating at 6 kV accelerating voltage with Xe atoms as the collision beam, and using a saturated solution of sodium acetate in 3-nitrobenzyl alcohol as the matrix unless otherwise stated. Specific rotations were run on a Perkin-Elmer 141 polarimeter as 1% w/v solutions in chloroform unless otherwise stated and IR spectra were collected on a Perkin-Elmer 197 IR spectrophotometer in chloroform solution. Solutions were dried using magnesium sulphate and solvents were removed by evaporation under reduced pressure using a rotary evap-

Table 9 Bond angles/° for **25** with esds in parentheses

O(1)–C(1)–C(2)	123.4(5)	O(1)–C(1)–O(13)	123.0(5)
C(2)–C(1)–O(13)	113.5(4)	C(1)–C(2)–C(3)	111.3(4)
C(1)–C(2)–C(16)	107.4(5)	C(3)–C(2)–C(16)	113.2(5)
C(2)–C(3)–O(3)	108.5(4)	C(2)–C(3)–C(4)	113.3(5)
O(3)–C(3)–C(4)	109.0(4)	C(3)–O(3)–C(17)	116.1(4)
C(3)–C(4)–C(5)	111.8(4)	C(3)–C(4)–C(41)	110.9(5)
C(5)–C(4)–C(41)	112.4(4)	C(4)–C(5)–O(5)	110.5(4)
C(4)–C(5)–C(6)	113.9(4)	O(5)–C(5)–C(6)	105.7(4)
C(5)–O(5)–C(25)	116.0(4)	C(5)–C(6)–O(6)	106.3(4)
C(5)–C(6)–C(7)	111.1(4)	O(6)–C(6)–C(7)	111.2(4)
C(5)–C(6)–C(33)	108.8(4)	O(6)–C(6)–C(33)	108.2(5)
C(7)–C(6)–C(33)	111.0(5)	C(6)–C(7)–C(8)	118.8(5)
C(7)–C(8)–C(9)	116.0(5)	C(7)–C(8)–C(34)	109.8(5)
C(9)–C(8)–C(34)	108.5(5)	C(8)–C(9)–N(9)	111.8(5)
C(8)–C(9)–C(10)	112.8(5)	N(9)–C(9)–C(10)	111.3(5)
C(9)–N(9)–C(35)	112.3(5)	C(9)–N(9)–C(38)	110.9(4)
C(35)–N(9)–C(38)	109.5(5)	C(9)–C(10)–C(11)	108.6(5)
C(9)–C(10)–C(36)	111.0(5)	C(11)–C(10)–C(36)	115.1(5)
C(10)–C(11)–O(11)	109.9(4)	C(10)–C(11)–C(12)	117.4(5)
O(11)–C(11)–C(12)	107.0(4)	C(11)–O(11)–C(38)	114.2(4)
C(11)–C(12)–O(12)	110.9(4)	C(11)–C(12)–C(13)	110.9(4)
O(12)–C(12)–C(13)	106.4(4)	C(11)–C(12)–C(37)	109.4(5)
O(12)–C(12)–C(37)	106.5(4)	C(13)–C(12)–C(37)	112.6(5)
C(12)–C(13)–O(13)	109.7(4)	C(12)–C(13)–C(14)	113.8(5)
O(13)–C(13)–C(14)	104.6(5)	C(1)–O(13)–C(13)	119.2(4)
C(13)–C(14)–C(15)	113.2(6)	O(3)–C(17)–O(17)	112.6(4)
O(3)–C(17)–C(18)	109.7(5)	O(17)–C(17)–C(18)	111.4(5)
C(17)–O(17)–C(21)	116.7(4)	C(17)–C(18)–C(19)	116.9(5)
C(18)–C(19)–O(19)	113.3(5)	C(18)–C(19)–C(20)	107.4(5)
O(19)–C(19)–C(20)	101.6(5)	C(18)–C(19)–C(23)	112.2(6)
O(19)–C(19)–C(23)	110.9(6)	C(20)–C(19)–C(23)	110.8(6)
C(19)–O(19)–C(22)	117.6(5)	C(19)–C(20)–O(20)	111.6(5)
C(19)–C(20)–C(21)	112.3(5)	O(20)–C(20)–C(21)	109.9(5)
O(17)–C(21)–C(20)	110.9(5)	O(17)–C(21)–C(24)	106.1(5)
C(20)–C(21)–C(24)	111.8(5)	O(5)–C(25)–O(25)	107.5(4)
O(5)–C(25)–C(26)	107.8(4)	O(25)–C(25)–C(26)	110.2(5)
C(25)–O(25)–C(29)	112.5(5)	C(25)–C(26)–O(26)	109.2(5)
C(25)–C(26)–C(27)	110.0(5)	O(26)–C(26)–C(27)	108.5(5)
C(26)–C(27)–C(28)	110.2(6)	C(26)–C(27)–N(27)	110.1(5)
C(28)–C(27)–N(27)	115.5(6)	C(27)–C(28)–C(29)	111.3(6)
O(25)–C(29)–C(28)	109.7(5)	O(25)–C(29)–C(30)	106.0(6)
C(28)–C(29)–C(30)	111.6(6)	C(27)–N(27)–C(31)	112.5(7)
C(27)–N(27)–C(32)	110.0(7)	C(31)–N(27)–C(32)	114.5(8)
N(9)–C(38)–O(11)	112.2(4)	N(9)–C(38)–C(39)	114.9(5)
O(11)–C(38)–C(39)	106.9(5)	C(38)–C(39)–O(40)	113.7(6)

orator with bath temperatures below 30 °C. Compounds were purified by column chromatography on silica-gel eluting with chloroform–methanol–0.880 ammonia (91:8:1) unless stated otherwise.

(9S)-6,9-O,N-9,11-N,O-*Bismethyleneerythromycylamine A* **14** *and* (9S)-9,11-N,O-*Methyleneerythromycylamine A* **11**.—(9*S*)-Erythromycylamine A (**5**, 500 mg) was dissolved in ethanol (5 cm^3) and 37% aqueous formaldehyde (1 cm^3) was added. The mixture was stirred at room temperature for 30 min, diluted with ethyl acetate (100 cm^3) and washed with water (3 × 20 cm^3). The organic layer was dried and evaporated to give **14** as a colourless foam; m.p. (light petroleum, b.p. 60–80 °C) 210–212 °C (softens *ca.* 160 °C) [lit.,[11] 215 °C (ether)] (Found: C, 61.1; H, 9.1; N, 3.4. Calc. for $C_{39}H_{70}N_2O_{12}$ 0.5 H_2O: C, 61.0; H, 9.3; N, 3.6); $[\alpha]_D^{20}$ −6.8°; mass spectrum *m/z* 758 (M^+), FAB-MS MNa^+ 781 ($C_{39}H_{70}N_2NaO_{12}$). Chromatography of the product **14** (300 mg) on silica-gel eluting with dichloromethane–methanol–0.880 ammonia (90:10:1) gave a white foam (250 mg) of R_f identical with that of **14** but which was shown to be the 6,9-mono adduct **11**; m.p. (needles from light petroleum b.p. 60–80 °C) 188–192 °C (softens 140 °C) (Found: C, 61.2; H, 9.4; N, 3.4. Calc. for $C_{38}H_{70}N_2O_{12}$: C, 61.1; H, 9.4; N, 3.7); $[\alpha]_D^{20}$ −35.6°; ν_{max}/cm^{-1} 1728; mass spectrum *m/z* 746 (M^+), FAB-MS MNa^+ 769 ($C_{38}H_{70}N_2NaO_{12}$).

Table 10 Atom coordinates (× 10^4) for **28** with estimated standard deviations (esds) in parentheses

Atom	*x*	*y*	*z*
O(1)	4 638(4)	−2 318(1)	1 097(1)
C(1)	3 959(4)	−1 825(2)	1 163(2)
C(2)	3 944(4)	−1 205(2)	805(2)
C(3)	5 142(3)	−728(2)	976(1)
O(3)	6 193(2)	−695(1)	570(1)
C(4)	4 642(4)	−9(2)	1 083(2)
C(5)	5 744(3)	466(2)	1 340(1)
O(5)	5 934(2)	1 050(1)	998(1)
C(6)	5 351(4)	739(2)	1 921(2)
O(6)	6 274(3)	1 247(1)	2 090(1)
C(7)	5 313(4)	1 73(2)	2 356(2)
C(8)	6 567(4)	−267(2)	2 434(2)
C(9)	6 418(4)	−784(2)	2 911(2)
N(9)	7 401(3)	−1 332(2)	2 822(1)
C(10)	4 979(4)	−1 056(2)	3 021(2)
C(11)	4 614(4)	−1 562(2)	2 574(2)
O(11)	5 596(3)	−2 094(1)	2 567(1)
C(12)	3 208(4)	−1 888(2)	2 569(2)
O(12)	3 077(3)	−2 363(1)	3 003(1)
C(13)	3 035(4)	−2 262(2)	2 017(2)
O(13)	3 085(2)	−1 751(1)	1 583(1)
C(14)	1 714(4)	−2 647(2)	1 955(2)
C(15)	1 583(6)	−3 040(3)	1 433(3)
C(16)	3 932(5)	−1 403(2)	198(2)
C(17)	7 076(4)	−1 266(2)	578(2)
O(17)	7 998(3)	−1 231(1)	1 013(1)
C(18)	7 751(5)	−1 320(2)	18(2)
C(19)	8 883(4)	−813(2)	−80(2)
O(19)	8 410(3)	−129(2)	−107(1)
C(20)	9 799(4)	−815(3)	427(2)
O(20)	10 795(3)	−303(2)	381(2)
C(21)	8 997(4)	−723(2)	962(2)
C(22)	7 431(8)	29(3)	−504(2)
C(23)	9 675(7)	−1 012(4)	−592(3)
C(24)	9 909(6)	−789(4)	1 460(3)
C(25)	7 242(3)	1 157(2)	790(1)
O(25)	8 120(2)	1 177(1)	1 256(1)
C(26)	72 11(4)	1 819(2)	478(1)
O(26)	6 516(3)	1 720(1)	−29(1)
C(27)	8 647(4)	2 062(2)	354(2)
N(27)	8 761(4)	2 731(1)	85(1)
C(28)	9 484(4)	2 056(2)	875(2)
C(29)	9 471(4)	1 356(2)	1 136(2)
C(30)	10 259(4)	1 323(3)	1 665(2)
C(31)	7 944(6)	3 256(2)	349(2)
C(32)	8 502(5)	2 725(2)	−508(2)
C(33)	3 965(4)	1 092(2)	1 911(2)
C(34)	7 862(4)	123(2)	2 542(2)
C(35)	8 036(5)	−1 606(3)	3 313(2)
C(36)	4 839(6)	−1 363(3)	3 605(2)
C(37)	2 113(4)	−1 353(2)	2 656(2)
C(38)	6 912(4)	−1 831(2)	2 429(2)
C(39)	7 837(4)	−2 430(2)	2 351(2)
C(40)	7 451(5)	−2 849(2)	1 870(2)
O(40)	8 214(3)	−3 451(2)	1 881(2)
C(41)	7 855(6)	−3 914(3)	1 480(3)
C(42)	8 611(10)	−4 549(3)	1 534(4)
C(43)	4 000(4)	305(2)	576(2)

(9S)-9-N,N-*Dimethylerythromycylamine A* (**7**).—The bis-adduct **14** (1.0 g) was dissolved in methanol (10 cm^3) and sodium cyanoborohydride (300 mg) was added. The reaction mixture was adjusted to pH 4.0 with 15% aqueous citric acid and stirred at ambient temperature for 4 h, poured into 10% aqueous potassium carbonate (50 cm^3) and extracted with ethyl acetate (3 × 50 cm^3). The organic layers were combined, dried and evaporated to give **7** as a white foam which was purified by

Table 11 Bond lengths/Å for **28** with esds in parentheses

O(1)–C(1)	1.197(5)	C(1)–C(2)	1.504(5)
C(1)–O(13)	1.349(5)	C(2)–C(3)	1.573(5)
C(2)–C(16)	1.529(6)	C(3)–O(3)	1.439(4)
C(3)–C(4)	1.531(5)	O(3)–C(17)	1.430(5)
C(4)–C(5)	1.573(5)	C(4)–C(43)	1.521(6)
C(5)–O(5)	1.437(4)	C(5)–C(6)	1.563(5)
O(5)–C(25)	1.410(4)	C(6)–O(6)	1.421(4)
C(6)–C(7)	1.542(5)	C(6)–C(33)	1.543(5)
C(7)–C(8)	1.531(5)	C(8)–C(9)	1.555(5)
C(8)–C(34)	1.524(5)	C(9)–N(9)	1.475(5)
C(9)–C(10)	1.550(6)	N(9)–C(35)	1.456(6)
N(9)–C(38)	1.460(5)	C(10)–C(11)	1.522(5)
C(10)–C(36)	1.555(6)	C(11)–O(11)	1.434(4)
C(11)–C(12)	1.537(5)	O(11)–C(38)	1.446(5)
C(12)–O(12)	1.421(5)	C(12)–C(13)	1.543(6)
C(12)–C(37)	1.531(6)	C(13)–O(13)	1.462(5)
C(13)–C(14)	1.525(6)	C(14)–C(15)	1.495(8)
C(17)–O(17)	1.402(5)	C(17)–C(18)	1.523(6)
O(17)–C(21)	1.417(5)	C(18)–C(19)	1.526(7)
C(19)–O(19)	1.433(6)	C(19)–C(20)	1.531(7)
C(19)–C(23)	1.525(8)	O(19)–C(22)	1.406(7)
C(20)–O(20)	1.422(6)	C(20)–C(21)	1.538(7)
C(21)–C(24)	1.520(8)	C(25)–O(25)	1.431(4)
C(25)–C(26)	1.514(5)	O(25)–C(29)	1.419(4)
C(26)–O(26)	1.428(4)	C(26)–C(27)	1.534(5)
C(27)–N(27)	1.483(5)	C(27)–C(28)	1.517(5)
N(27)–C(31)	1.467(6)	N(27)–C(32)	1.466(5)
C(28)–C(29)	1.524(6)	C(29)–C(30)	1.506(6)
C(38)–C(39)	1.511(6)	C(39)–C(40)	1.485(7)
C(40)–O(40)	1.414(6)	O(40)–C(41)	1.385(7)
C(41)–C(42)	1.469(9)		

Table 12 Bond angles/° for **28** with esds in parentheses

O(1)–C(1)–C(2)	126.3(4)	O(1)–C(1)–O(13)	123.5(4)
C(2)–C(1)–O(13)	110.2(3)	C(1)–C(2)–C(3)	109.2(3)
C(1)–C(2)–C(16)	110.6(3)	C(3)–C(2)–C(16)	114.5(3)
C(2)–C(3)–O(3)	113.3(3)	C(2)–C(3)–C(4)	110.9(3)
O(3)–C(3)–C(4)	108.0(3)	C(3)–O(3)–C(17)	113.5(3)
C(3)–C(4)–C(5)	113.4(3)	C(3)–C(4)–C(43)	112.2(3)
C(5)–C(4)–C(43)	111.8(3)	C(4)–C(5)–O(5)	109.9(3)
C(4)–C(5)–C(6)	113.1(3)	O(5)–C(5)–C(6)	106.2(2)
C(5)–O(5)–C(25)	116.8(2)	C(5)–C(6)–O(6)	110.2(3)
C(5)–C(6)–C(7)	112.1(3)	O(6)–C(6)–C(7)	109.4(3)
C(5)–C(6)–C(33)	111.4(3)	O(6)–C(6)–C(33)	105.0(3)
C(7)–C(6)–C(33)	108.5(3)	C(6)–C(7)–C(8)	118.6(3)
C(7)–C(8)–C(9)	112.9(3)	C(7)–C(8)–C(34)	114.8(3)
C(9)–C(8)–C(34)	106.5(3)	C(8)–C(9)–N(9)	108.1(3)
C(8)–C(9)–C(10)	116.4(3)	N(9)–C(9)–C(10)	112.3(3)
C(9)–N(9)–C(35)	116.1(3)	C(9)–N(9)–C(38)	111.9(3)
C(35)–N(9)–C(38)	115.5(4)	C(9)–C(10)–C(11)	109.0(3)
C(9)–C(10)–C(36)	112.1(4)	C(11)–C(10)–C(36)	111.9(4)
C(10)–C(11)–O(11)	109.2(3)	C(10)–C(11)–C(12)	119.9(3)
O(11)–C(11)–C(12)	108.1(3)	C(11)–O(11)–C(38)	110.7(3)
C(11)–C(12)–O(12)	110.7(3)	C(11)–C(12)–C(13)	108.0(3)
O(12)–C(12)–C(13)	108.6(3)	C(11)–C(12)–C(37)	110.8(3)
O(12)–C(12)–C(37)	106.9(3)	C(13)–C(12)–C(37)	111.8(3)
C(12)–C(13)–O(13)	107.1(3)	C(12)–C(13)–C(14)	115.0(4)
O(13)–C(13)–C(14)	107.6(3)	C(1)–O(13)–C(13)	119.7(3)
O(13)–C(14)–C(15)	114.8(4)	O(3)–C(17)–O(17)	111.8(3)
O(3)–C(17)–C(18)	108.3(3)	O(17)–C(17)–C(18)	113.2(3)
C(17)–O(17)–C(21)	115.1(3)	C(17)–C(18)–C(19)	114.6(4)
C(18)–C(19)–O(19)	112.7(4)	C(18)–C(19)–C(20)	108.1(4)
O(19)–C(19)–C(20)	103.6(4)	C(18)–C(19)–C(23)	109.7(4)
O(19)–C(19)–C(23)	112.1(4)	C(20)–C(19)–C(23)	110.5(4)
C(19)–O(19)–C(22)	117.9(4)	C(19)–C(20)–O(20)	110.4(4)
C(19)–C(20)–C(21)	112.0(4)	O(20)–C(20)–C(21)	110.0(4)
O(17)–C(21)–C(20)	110.7(4)	O(17)–C(21)–C(24)	106.6(4)
C(20)–C(21)–C(24)	110.9(4)	O(5)–C(25)–O(25)	106.3(3)
O(5)–C(25)–C(26)	107.0(3)	O(25)–C(25)–C(26)	112.7(3)
C(25)–O(25)–C(29)	114.8(3)	C(25)–C(26)–O(26)	109.0(3)
C(25)–C(26)–C(27)	110.5(3)	O(26)–C(26)–C(27)	108.8(3)
C(26)–C(27)–N(27)	115.9(3)	C(26)–C(27)–C(28)	110.1(3)
N(27)–C(27)–C(28)	109.6(3)	C(27)–N(27)–C(31)	113.3(3)
C(27)–N(27)–C(32)	114.5(3)	C(31)–N(27)–C(32)	109.9(3)
C(27)–C(28)–C(29)	110.5(3)	O(25)–C(29)–C(28)	108.7(3)
O(25)–C(29)–C(30)	107.7(3)	C(28)–C(29)–C(30)	113.1(4)
N(9)–C(38)–O(11)	113.0(3)	N(9)–C(38)–C(39)	114.3(3)
O(11)–C(38)–C(39)	107.3(3)	C(38)–C(39)–C(40)	112.3(4)
C(39)–C(40)–O(40)	108.5(4)	C(40)–O(40)–C(41)	113.9(4)
O(40)–C(41)–C(42)	111.7(6)		

Table 13 Torsion angles/° around the tetrahydro-1,3-oxazine ring

Torsion angle	**25**	**28**	**10**
C(9)–C(10)–C(11)–O	64.4	57.5	57.4
C(10)–C(11)–O–C(23)	−43.6	−62.9	−57.7
C(11)–O–C(23)–N	−18.8	61.0	54.5
O–C(23)–N–C(9)	61.9	−52.9	−51.9
C(23)–N–C(9)–C(10)	−38.1	48.3	52.3
N–C(9)–C(10)–C(11)	−21.0	−50.7	−54.7
C(22)–N–C(23)–CH_2	−52.5	−40.1	—
C(8)–C(9)–C(10)–C(22)	−127.7	−160.6	−163.7
C(22)–N–C(9)–C(8)	72.3	142.8	—
C(12)–C(11)–C(10)–C(22)	62.7	58.0	55.5

column chromatography (832 mg). Recrystallisation from ethyl acetate gave needles, m.p. 185–187 °C (softens at 130 °C) (Found: C, 61.6; H, 9.9; N, 3.7. Calc. for $C_{39}H_{74}N_2O_{12}$: C, 61.4; H, 9.8; N, 3.7); $[\alpha]_D^{20}$ −52.3°; ν_{max}/cm^{-1} 3475 and 1725; *m/z* FAB-MS MNa^+ 785 ($C_{39}H_{74}N_2NaO_{12}$).

(9S)-9-N-*Methylerythromycylamine A* (**6**).—(9*S*)-9,11-Methyleneerythromycylamine A **11** (2.76 g) was treated for 2 h with sodium cyanoborohydride (0.89 g) in methanol (55 cm^3) in a procedure identical with that described for the preparation of **7**. Column chromatography gave **6** as a foam (2.1 g). Recrystallisation from ethyl acetate gave needles, m.p. 130–132 °C (softens 126 °C) (Found: C, 61.1; H, 9.8; N, 3.6. Calc. for $C_{38}H_{72}N_2O_{12}$: C, 60.9; H, 9.7; N, 3.5); $[\alpha]_D^{20}$ −45.5°; ν_{max}/cm^{-1} 3440 and 1710; *m/z* FAB-MS (thioglycerol matrix) MH^+ 749 ($C_{38}H_{73}N_2O_{12}$); FAB-MS MNa^+ 771 ($C_{38}H_{72}N_2NaO_{12}$).

(9S)-9-N-*Ethylerythromycylamine A* (**8**).—(9*S*)-Erythromyclamine A (**5**) (500 mg) was dissolved in ethanol (5 cm^3), acetaldehyde (1 cm^3) was added, and the reaction mixture was stirred at room temperature for 2 h and then evaporated. Rapid chromatography gave (9*S*)-9,11-*N*,*O*-ethylideneerythromycylamine A (**12**) (432 mg) (mixture of diastereoisomers) as an unstable foam which was treated for 1 h with sodium cyanoborohydride (158 mg) in methanol (5 cm^3) in a procedure identical with that described for the preparation of **7**. The product was chromatographed to give **8** (293 mg) as a white foam; $[\alpha]_D^{20}$ −42.8°; ν_{max}/cm^{-1} 3310 and 1708; *m/z* FAB-MS MNa^+ 785 ($C_{39}H_{74}N_2NaO_{12}$).

(9S)-9-N-(2-*Hydroxyethyl*)*erythromycylamine A* (**9**).—(9*S*)-Erythromycylamine A (**5**) (500 mg) was dissolved in THF (6 cm^3), glycolaldehyde dimer (150 mg) was added and the mixture was stirred at room temperature for 30 min. The solution was poured into 10% aqueous potassium carbonate (20 cm^3) and extracted with ethyl acetate (3 × 30 cm^3). The organic layers were combined, dried and evaporated to give a white foam (486 mg) which was unstable to chromatography but which gave a mass spectrum; FAB-MS MNa^+ 799 ($C_{39}H_{72}N_2NaO_{13}$) which was compatible with the expected structure (9*S*)-9,11-*N*,*O*-hydroxyethylideneerythromycylamine A (**13**). This product was taken without purification and treated for 3 h with sodium cyanoborohydride (150 mg) in methanol (8 cm^3) in a procedure identical with that described for the preparation of **7**. The product was chromatographed to give **9** (310 mg) (Found: C,

59.2; H, 9.5; N, 3.7. Calc. for $C_{39}H_{74}N_2O_{13}0.5H_2O$: C, 59.4; H, 9.6; N, 3.6); $[\alpha]^{20}$ −38.9° (1.2% w/v in $CHCl_3$); ν_{max}/cm^{-1} 1705; *m/z* FAB-MS MNa^+ 801 ($C_{39}H_{74}N_2NaO_{13}$).

(9S)-9-N-*Methyl*-9,11-N,O-*methyleneerythromycylamine A* **16**.—(9*S*)-9-*N*-Methylerythromycylamine A (**6**) (200 mg) was dissolved in ethanol (2 cm^3), 37% aqueous formaldehyde (0.5 cm^3) was added, and the mixture was stirred at ambient temperature for 30 min and then evaporated. The resulting foam was chromatographed to remove the formaldehyde residues, and gave **16** (165 mg) (Found: C, 60.1; H, 9.3; N, 3.6. Calc. for $C_{39}H_{72}N_2O_{12}\cdot H_2O$: C, 60.1; H, 9.6; N, 3.6); $[\alpha]^{20}$ −46.8°; ν_{max}/cm^{-1} 1732; *m/z* FAB-MS MNa^+ 783 ($C_{39}H_{72}N_2NaO_{12}$); δ_C(223 K) C(1)–C(23) 175.0, 44.7, 79.4, 38.6, 83.9, 73.4, 34.7, 29.1, 73.3, 28.8, 70.1, 74.0, 75.1, 21.0, 10.8, 16.6, 9.5, 28.0, 21.0, 20.7, 15.5, 38.4, 82.1; C(1′)–C(7′ + 8′) 102.9, 70.7, 65.2, 28.5, 68.8, 21.7, 40.5; C(1″)–C(8″) 95.8, 34.0, 72.6, 77.7, 65.2, 18.7, 21.7, 49.8; δ_C(323 K) C(1)–C(23) 175.5, 44.8, 79.2, 44.7, 83.2, 73.9, 36.5, 29.7, 73.9, 29.2, 71.5, 74.3, 76.1, 21.4, 10.8, 14.9, 9.1, 26.4, 20.7, 19.0, 15.4, 40.0, 82.5; C(1′)–C(7′ + 8′) 102.6, 71.3, 65.4, 29.3, 68.9, 21.2, 40.3; C(1″)–C(8″) 95.6, 35.0, 72.8, 78.2, 65.8, 18.4, 21.6, 49.3 (assignments provisional only).

General Procedure for the Preparation of Compounds **21–28**.—(9*S*)-9-*N*-Methylerythromycylamine A (**6**) was dissolved in ethanol or, preferably, dry THF, the aldehyde was added and the solution was stirred at ambient temperature for the indicated time (Table 2) and then poured into 10% aqueous potassium carbonate and extracted with ethyl acetate (×3). [For **21** the reaction mixture was simply evaporated prior to chromatography while for **23**, **24** and **26**, the reaction mixture was diluted with water and acidified to pH 3 with hydrochloric acid (2 mol dm^{-3}). The aqueous solution was extracted with ether (×3) which was discarded (to remove the excess aldehyde). The aqueous residue was basified to pH 11 by the addition of 10% aqueous potassium carbonate and then extracted with ethyl acetate (×3).] The organic layers were combined, dried and evaporated to give a white foam which was purified by column chromatography.

Table 14 Torsion angles[16]/° for cyclohexane conformations

Torsion angle	Chair	Boat	Twist-boat
C(1)–C(2)–C(3)–C(4)	60	60	70.6
C(2)–C(3)–C(4)–C(5)	−60	0	−33.2
C(3)–C(4)–C(5)–C(6)	60	−60	−33.2
C(4)–C(5)–C(6)–C(1)	−60	60	70.6
C(5)–C(6)–C(1)–C(2)	60	0	−33.2
C(6)–C(1)–C(2)–C(3)	−60	−60	−33.2

(9S,23RS)-9-N-*Methyl*-9,11-N,O-*ethylideneerythromycylamine A* (**21**). The product (155 mg) was shown by HPLC and TLC [on silica-gel, eluting with chloroform–methanol–0.880 ammonia (91:8:1)] to be a mixture of two closely running compounds **21** (diastereoisomers about C-23), which could not be separated. The mixture had the following physical properties; $[\alpha]_D^{20}$ −45.5°; ν_{max}/cm^{-1} 1722; *m/z* FAB-MS (3-nitrobenzyl alcohol matrix) MH^+ 775 ($C_{40}H_{75}N_2O_{12}$); FAB-MS MNa^+ 797 ($C_{40}H_{74}N_2NaO_{12}$).

(9S,23RS)-9-N-*Methyl*-9,11-N,O-*propylideneerythromycylamine A* (**22**). The product was shown by HPLC and TLC to be a mixture of two closely running compounds **22** which could not be isolated in pure form and which were diastereoisomeric about C-23. The mixture had the following physical properties: ν_{max}/cm^{-1} 1720; *m/z* FAB-MS MNa^+ 811 ($C_{41}H_{76}N_2NaO_{12}$).

(9S,23S)-9-N-*Methyl*-9,11-N,O-(2-*phenylethylidine*)*erythromycylamine A* (**23**), *and its* (23R) *isomer* **24**. The product was shown by chromatography to be a mixture of two separable compounds in the approximate ratio of 1:3 (less polar:more polar). Less polar isomer **23** (52 mg); $[\alpha]_D^{20}$ −72.5°; ν_{max}/cm^{-1} 1722; *m/z* FAB-MS (3-nitrobenzyl alcohol matrix MH^+ 851 ($C_{46}H_{79}N_2O_{12}$); FAB-MS MNa^+ 873 ($C_{46}H_{78}N_2NaO_{12}$). More polar isomer **24** (123 mg); $[\alpha]_D^{20}$ −64.0°; ν_{max}/cm^{-1} 1722; *m/z* FAB-MS (3-nitrobenzyl alcohol matrix), MH^+ 851 ($C_{46}H_{79}N_2O_{12}$); FAB-MS MNa^+ 873 ($C_{46}H_{78}N_2NaO_{12}$). The more polar isomer in chloroform at 50 °C isomerised into the less polar isomer to give a *ca.* 1:1 mixture after 16 h.

(9S,23S)-9-N-*Methyl*-9,11-N,O-(2-*hydroxyethylidene*)*erythromycylamine A* (**25**). The product was shown by chromatography and HPLC to be predominantly one compound with a small amount of a more polar product present. The ratio was *ca.* 8:1 (less polar:more polar). Chromatography gave **25** (1.82 g) which was recrystallised from ethyl acetate; m.p. 150–155 °C (softens 145 °C) (Found: C, 59.6; H, 9.6; N, 3.6. Calc. for $C_{40}H_{74}N_2O_{13}\cdot H_2O$: C, 59.4; H, 9.5; N, 3.5); $[\alpha]_D^{20}$ −53.3°; ν_{max}/cm^{-1} 3545, 3490 and 1725; *m/z* FAB-MS MNa^+ 813 ($C_{40}H_{74}N_2NaO_{13}$). The minor (more polar) component could not be isolated in a pure state.

(9S,23R)-9-N-*Methyl*-9,11-N,O-(2,2-*diphenylethylidene*)-*erythromycylamine A* (**26**). The product was shown by TLC to be mainly one compound with a small amount of a less polar compound. The ratio of less polar:more polar was *ca.* 1:25 by HPLC. The major product **26** was isolated pure (211 mg) by a combination of reversed-phase column chromatography (silanised silica-gel, eluting with methanol:pH 7.0 phosphate buffer 3:2) and normal-phase column chromatography (Found: C, 66.6; H, 9.0; N, 2.9. Calc. for $C_{52}H_{82}N_2O_{12}\cdot 0.5H_2O$: C, 66.7; H, 8.9; N, 3.0); $[\alpha]_D^{20}$ +1.8°; ν_{max}/cm^{-1} 3520 and 1722; *m/z* FAB-MS (3-nitrobenzyl alcohol matrix) MH^+ 927 ($C_{52}H_{83}N_2O_{12}$); FAB-MS MNa^+ 949 ($C_{52}H_{82}N_2NaO_{12}$).

Table 15 Torsion angles/° around the erythronolide ring

Torsion angle	1	4	10	25	28
O–C(1)–C(2)–C(3)	115.9	79.9	94.4	90.9	97.0
C(1)–C(2)–C(3)–C(4)	−61.2	−130.8	−129.6	−66.4	−128.2
C(2)–C(3)–C(4)–C(5)	164.5	173.6	172.8	169.2	171.4
C(3)–C(4)–C(5)–C(6)	−116.1	−100.1	−116.9	−84.5	−118.3
C(4)–C(5)–C(6)–C(7)	−68.5	60.1	67.6	−75.6	67.5
C(5)–C(6)–C(7)–C(8)	175.0	148.0	55.5	175.0	54.9
C(6)–C(7)–C(8)–C(9)	−77.0	178.9	177.4	−90.5	176.2
C(7)–C(8)–C(9)–C(10)	−60.8	−53.9	34.5	−55.6	31.4
C(8)–C(9)–C(10)–C(11)	122.0	97.2	70.3	105.1	74.7
C(9)–C(10)–C(11)–C(12)	−173.3	−164.0	−156.0	−172.3	−177.3
C(10)–C(11)–C(12)–C(13)	167.8	165.6	173.6	177.8	169.1
C(11)–C(12)–C(13)–O	−68.6	−70.1	−59.5	−61.9	−63.5
C(12)–C(13)–O–C(1)	107.3	144.6	156.0	113.9	126.6
C(13)–O–C(1)–C(2)	171.3	−177.9	−173.6	170.1	−173.5

(9S,23S)-9-N-*Methyl*-9,11-N,O-(3-*ethoxypropylidene*)-*erythromycylamine A* (**27**) *and its* (23R) *isomer* **28**. The product was a mixture of two compounds which were separated by column chromatography. The less polar isomer **27** (37 mg) (Found: C, 62.2; H, 9.5; N, 3.3. Calc. for $C_{43}H_{80}N_2O_{13}$: C, 62.0; H, 9.7; N, 3.4); $[\alpha]_D^{20}$ −66.8°; ν_{max}/cm^{-1} 1732; *m/z* FAB-MS MNa^+ 855 ($C_{43}H_{80}N_2NaO_{13}$). The more polar isomer **28** (87 mg); m.p. (ethyl acetate) 209–213 °C (Found: C, 61.8; H, 9.7; N, 3.4. Calc. for $C_{43}H_{80}N_2O_{13}$: C, 62.0; H, 9.7; N, 3.4); $[\alpha]_D^{20}$ −59.3°; ν_{max}/cm^{-1} 1730; *m/z* FAB-MS MNa^+ 855 ($C_{43}H_{80}N_2NaO_{13}$).

When the less polar isomer **27** was crystallised from hot ethyl acetate it gave data identical with that shown by the more polar isomer **28**.

X-Ray Diffraction Studies.—Much of the detail is common to both studies, therefore only the differences from **25** are reported for **28**. Similarly, for the crystal data parameters.

Crystal data. Compound **25**: $C_{40}H_{74}N_2O_{13}\cdot 3H_2O$, M = 845.1, orthorhombic, a = 20.742(8), b = 21.166(8), c = 23.878(13) Å, U = 10 483 Å^3, space group $I222$, Z = 8, D_c = 1.07 g cm^{-3}, Cu radiation, λ = 1.541 78 Å, μ(Cu-Kα) = 6 cm^{-1}, $F(000)$ = 3696.

Compound **28**: $C_{43}H_{80}N_2O_{13}$, M = 833.1, orthorhombic, a = 9.931(2), b = 19.789(5), c = 24.343(6) Å, U = 4784 Å^3, space group $P2_12_12_1$, Z = 4, D_c = 1.16 g cm^{-3}, μ(Cu-Kα) = 7 cm^{-1}, $F(000)$ = 1824.

Data Collection and Structure Analysis.—Compound **25**. Data were measured on a Nicolet R3m diffractometer with Cu-Kα radiation (graphite monochromator) using ω-scans. 3880 independent reflections were measured ($2\theta \leqslant 116°$), of which 3323 had $|F_o| > 3\sigma(|F_o|)$ and were considered to be observed. The data were corrected for Lorentz and polarisation factors; no absorption correction was applied. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. A ΔF map revealed the presence of six half-weight water molecules, the hydrogen atoms of which were not located. The hydroxy protons on O(6), O(12), O(20), O(26) and O(40) were located from a ΔF map and refined isotropically. The positions of the remaining hydrogen atoms were idealised, C–H = 0.96 Å, assigned isotropic thermal parameters, U(H) = 1.2 U_{eq}(C), and allowed to ride on their parent carbon atoms. The methyl groups were refined as rigid bodies. Refinement was by block-cascade, full-matrix least-squares to R = 0.070, R_w = 0.068 [$w^{-1} = \sigma^2(F) + 0.000\,50F^2$]. The maximum and minimum residual electron densities in the final ΔF map were 0.55 and −0.24 e Å^{-3}, respectively. The mean and maximum shift/error in the final refinement were 0.021 and 0.288, respectively. Computations were carried out on an Eclipse S140 computer using the SHELXTL program system.[23]

Fractional atomic coordinates for the non-hydrogen atoms are given in Table 7. Tables 8 and 9 list bond lengths and bond angles.*

Compound **28**. 3526 independent reflections were measured ($2\theta \leqslant 116°$), of which 3239 had $|F_o| > 3\sigma(|F_o|)$ and were considered to be observed. The structure was solved by direct methods. The hydroxy protons on O(6), O(12), O(20) and O(26) were located from a ΔF map and refined isotropically. Convergence occurred at R = 0.044, R_w = 0.051 using a weighting scheme of the form [$w^{-1} = \sigma^2(F) + 0.00\,114F^2$]. The maximum and minimum residual electron densities in the final ΔF map were 0.20 and 0.15 e Å^{-3} respectively. The mean and maximum shift/error in the final refinement were 0.016 and 0.087, respectively.

Fractional atomic coordinates for the non-hydrogen atoms are given in Table 10. Tables 11 and 12 list bond lengths and bond angles respectively.*

* Full lists of hydrogen atom coordinates, temperature parameters and torsion angles for compounds **25** and **28** have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme see 'Instructions for Authors (1991),' *J. Chem. Soc., Perkin Trans. 2*, in the January issue.

Acknowledgements

The authors would like to thank Dr. J. H. C. Nayler and Mr. A. E. Bird for their interest in this work. Our thanks are also due to our colleagues in the Physical and Analytical Services Unit at Brockham Park for MS and CHN data and to Miss J. Goddard for expert typing.

References

1 P. Kurath, P. H. Jones, R. S. Egan and T. J. Perun, *Experientia*, 1971, **27**, 362.
2 (*a*) S. Djokic and Z. Tamburasev, *Tetrahedron Lett.*, 1967, 1645; (*b*) E. H. Massey, B. Kitchell, L. D. Martin, K. Gerzon and H. W. Murphy, *Tetrahedron Lett.*, 1970, 157.
3 USP No. 3,681,326 (filed 11,1,71 issued 1.8.72) (*Chem. Abstr.*, 1972, **77**, 114273m).
4 (*a*) G. H. Timms and E. Wildsmith, *Tetrahedron Lett.*, 1971, 195; (*b*) E. Wildsmith, *Tetrahedron Lett.*, 1972, 29.
5 W. Slawinski, H. Bojarska-Dahlig, T. Glabski, I. Dziegielewska, M. Biedrzycki and S. Naperty, *Recl. Trav. Chim. Pays-Bas*, 1975, **94**, 236.
6 Y. Watanabe, T. Adachi, S. Morimoto, S. Namiki, S. Ōmura and J. Sawada, Jpn. Kokai 70293, June 5th 1979 (*Chem. Abstr.*, 1980, **92**, 6879q).
7 T. Adachi, Y. Watanabe, S. Morimoto, S. Ōmura and J. Sawada, Jpn. Kokai 144589 Dec. 15th, 1978 (*Chem. Abstr.*, 1979, **90**, 152554e).
8 E. Hunt, D. J. C. Knowles, C. Shillingford and I. I. Zomaya, *J. Antibiot.*, 1988, **41**, 1644.
9 E. Hunt, D. J. C. Knowles, C. Shillingford, J. M. Wilson and I. I. Zomaya, *J. Antibiot.*, 1989, **42**, 293.
10 R. Ryden, G. H. Timms, D. M. Prime and E. Wildsmith, *J. Med. Chem.*, 1973, **16**, 1059.
11 E. H. Massey, B. S. Kitchell, L. D. Martin and K. Gerzon, *J. Med. Chem.*, 1974, **17**, 105.
12 USP, 4,048,306, 1977.
13 P. Luger and R. Maier, *J. Cryst. Mol. Struct.*, 1979, **9**, 329.
14 Eli Lilly and Company, European Patent, EP 238,178 (23.9.87).
15 J. R. Everett and J. W. Tyler, *J. Chem. Soc., Perkin Trans. 2*, 1988, 325.
16 J. R. Everett and J. W. Tyler, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1659.
17 J. R. Everett, I. K. Hatton, E. Hunt, J. W. Tyler and D. J. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1719.
18 J. R. Everett and J. W. Tyler, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2599.
19 J. C. Christofides and D. B. Davies, *J. Chem. Soc., Chem. Commun.*, 1982, 560.
20 J. B. Lambert and M. W. Majchrzak, *J. Am. Chem. Soc.*, 1980, **102**, 3588; G. Bernáth, F. Fülöp, A. Kálmán, G. Argay, P. Sohár and I. Pelczer, *Tetrahedron*, 1984, **40**, 3587; F. Fülöp, K. Pihlaja and G. Bernáth, *Acta Chem. Scand., Ser. B*, 1987, **41**, 147; F. Fülöp, K. Pihlaja, J. Mattinen and G. Bernáth, *J. Org. Chem.*, 1987, **52**, 3821.
21 J. B. Hendrickson, *J. Am. Chem. Soc.*, 1961, **83**, 4537.
22 (*a*) D. R. Harris, S. G. McGeachin and H. H. Mills, *Tetrahedron Lett.*, 1965, 679; (*b*) A. Hempel, M. Bogucka-Ledochowska, Z. Dauter, E. Borowski and Z. Kosturkiewicz, *J. Cryst. Mol. Struct.*, 1975, **5**, 387; (*c*) A. Hempel, *Acta Crystallogr., Sect. B*, 1978, **34**, 3454; (*d*) P. Barner, D. L. Boxier, R. Brambilla, A. K. Mallams, J. B. Morton, P. Reichert, F. D. Sancilio, H. Surprenant, G. Tomalesky, G. Lukacs, A. Olesker, T. T. Thang, L. Valente and S. Omura, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1600; (*e*) B. Bachet, C. Brassy and J.-P. Mornon, *Acta Crystallogr., Sect. C*, 1988, **44**, 112.
23 G. M. Sheldrick, SHELXTL, An integrated system for solving, refining and displaying crystal structures from diffraction data, University of Göttingen, Federal Republic of Germany, 1978; Revision 4.1, 1983.

Paper 0/03217D
Received 17*th July* 1990
Accepted 2*nd October* 1990