Ketone–Hemiacetal Tautomerism in Erythromycin A in Non-aqueous Solutions. An NMR Spectroscopic Study

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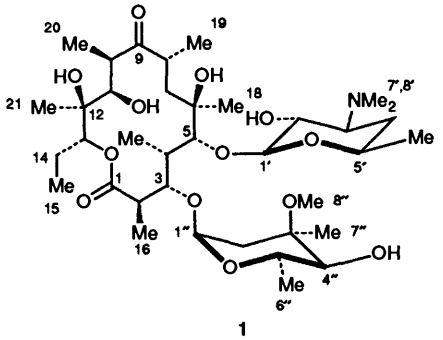
NMR spectroscopic studies, including ^{13}C SIMPLE NMR, in a number of solvents, show that erythromycin A exists as a mixture of the 9-ketone (the predominant species), one 6,9-cyclic hemiacetal **3** (9-deoxo-6-deoxy-9-hydroxy-6,9-epoxyerythromycin A), and one 9,12-cyclic hemiacetal **2** (9-deoxo-12-deoxy-9-hydroxy-9,12-epoxyerythromycin A). Similar studies on the 4″,11-diacetate of erythromycin A, previously thought to be the 6,9-cyclic hemiacetal, show that this exists exclusively as the 9,12-cyclic hemiacetal **4** (9-deoxo-12-deoxy-9-hydroxy-9,12-epoxyerythromycin A 4″,11-diacetate) in $CDCl_3$. The (9*S*) stereochemistry is proposed for **2**, **3** and **4**. The factors governing tautomerism in erythromycin A and its derivative are discussed.

Erythromycin A **1**† is one of the most important members of the macrolide group of antibiotics,[1] and has been used in medicine for almost four decades.[2] During recent years, there has been a revival of interest in this class of compounds, and in erythromycin in particular,[3] and an integral part of this



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renaissance has been the detailed study of these molecules using modern methods of NMR spectroscopy. Thus, the first unambiguous assignments of the ^1H and ^{13}C NMR spectra of erythromycin A in deuteriochloroform were published from these laboratories six years ago,[4] and subsequent reports have described the solution-state conformational analysis of erythromycin[5] and some of its derivatives.[6] We now report an important extension to these studies, wherein we describe an NMR spectroscopic investigation into the ketone–hemiacetal tautomerism‡ of erythromycin A in non-aqueous solution.§

The ^1H and ^{13}C NMR spectra of erythromycin A in $CDCl_3$ show that in this solvent the compound exists predominantly as the C-9 ketone **1**.[8] However, the spectra also show weak signals which indicate that two other species are present,[4,5] and a preliminary examination of the ^{13}C NMR resonances for these minor components suggested that they are 6,9- or 9,12-cyclic hemiacetal tautomers of **1**.¶ For a more detailed examination of these structures we needed a solvent in which the hemiacetal signals were more pronounced. Measurement of the ^{13}C NMR spectra in five other solvents (Table 1) showed that the hemiacetal signals were appreciably stronger in CD_3OD, $(CD_3)_2NCDO$ ($[^2H_7]$DMF), and $(CD_3)_2SO$ ($[^2H_6]$DMSO), than in $CDCl_3$.

The ^1H and ^{13}C NMR spectra of erythromycin A in CD_3OD, $[^2H_6]$DMSO, and $[^2H_7]$DMF were studied using the one- and two-dimensional methods previously employed for this type of molecule.[4–6] For example, for all three tautomers in $[^2H_6]$DMSO, C-5 and 5-H were assigned unambiguously using 1D ^1H and ^{13}C, 2D ^1H COSY-45, and 2D ^1H, ^{13}C COSY experiments; these assignments then gave that of 18-H, by way of a 2D ^1H, ^{13}C COLOC experiment[9] (correlation by long range coupling, tuned for 2–4 bond interactions) (Fig. 1), and hence assignments of C-6 (by way of 2D ^1H, ^{13}C COLOC) and C-18 (by way of 2D ^1H, ^{13}C COSY). Similarly, on the other side of the macrocycle, unambiguous assignments of C-13 and 13-H allowed 21-H and then C-12 to be assigned for all three compounds, and C-21 to be assigned for the ketone and major hemiacetal.‖ The remaining peaks in the spectra were similarly analysed to give complete spectral assignments for the ketone and major hemiacetal, and a substantial number of assignments for the minor hemiacetal (Table 2). Importantly, the unambiguous identification of all the C-6 and C-12 resonances allowed us to distinguish between the two possible structures for the hemiacetals: the major hemiacetal (C-6, δ74.1; C-12, δ84.2) was assigned the 9,12-cyclic structure **2**, and the minor hemiacetal (C-6, δ83.5; C-12, δ74.3) was assigned the 6,9-cyclic structure **3**.

Further confirmation of these structural assignments was

† The erythromycin structure consists of a 14-membered lactone aglycone, erythronolide, which carries an amino sugar, D-desosamine, and a neutral sugar, L-cladinose. Unprimed numbers are used for positions in the aglycone, primed numbers are used in the amino sugar, and double primed numbers are used in the neutral sugar. In structural formulae we use the abbreviations Cla for α-L-cladinosyl and Des for β-D-desosaminyl.

‡ The ketone and hemiacetal forms are ring-chain tautomers. The abbreviated term 'tautomer' is used for 'ring-chain tautomer' in this paper.

§ An independent, contemporaneous study of the ketone–hemiacetal tautomerism in D_2O and $(CD_3)_2SO$ is described by Barber, *et al.* (ref. 7). The major findings from this study are in agreement with our own.

¶ In particular, the minor components show no ketonic carbonyl signals, but signals at δ 110.5 and 107.8 which were assigned to C-9 acetal-type carbons, and signals for quaternary carbons at δ87.3 and 84.6 which were assigned to C-6/C-12 carbons bearing ether-type oxygens.

‖ C-21 could not be assigned unambiguously for the minor hemiacetal owing to spectral crowding and the presumed relative weakness of this signal.

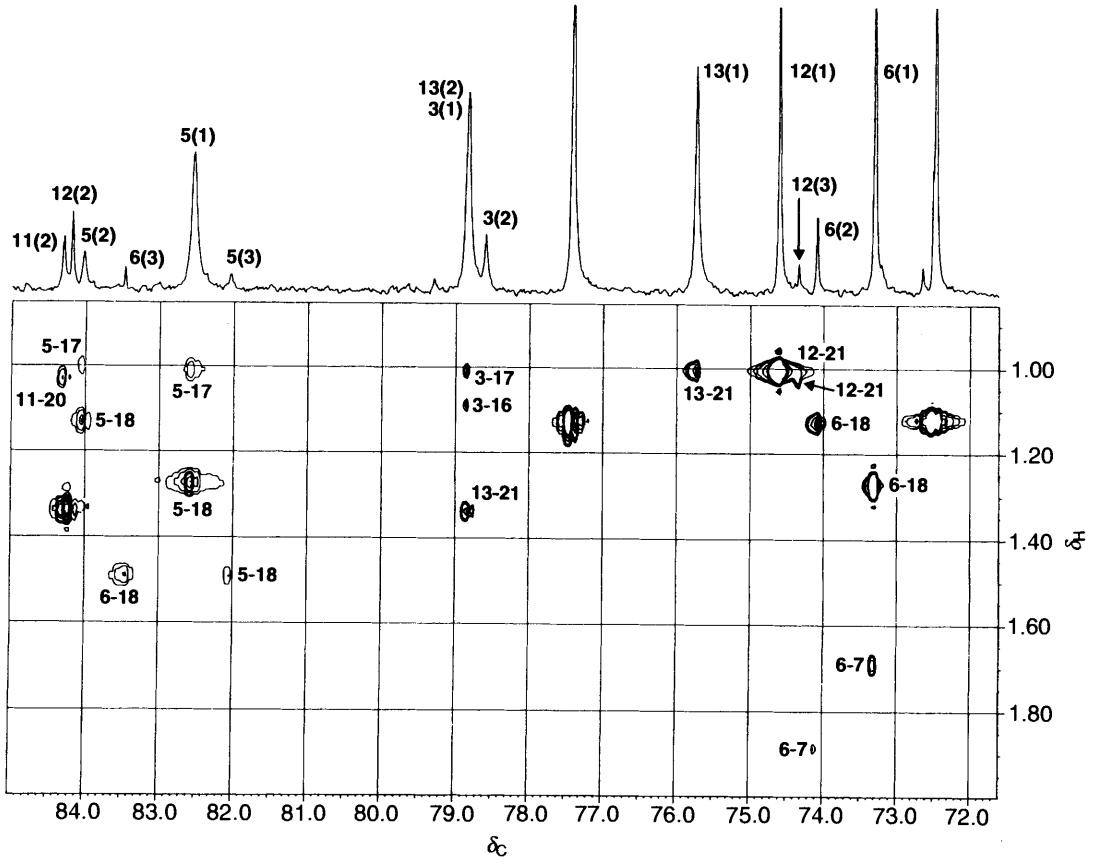
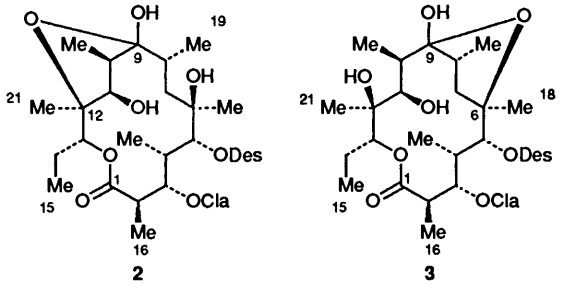


Fig. 1 Contour plot of part of the ^1H, ^{13}C COLOC spectrum of erythromycin A in $(CD_3)_2SO$–Me_4Si at 27 °C. Proton–carbon correlations giving rise to the cross-peaks are indicated, for tautomers **1**, **2** and **3**. Peaks in the ^{13}C spectrum are labelled with the carbon number, with the tautomer indicated in parentheses.

Table 1 Relative proportions of the tautomeric forms of erythromycin A in various solvents at 27 °C

Solvent	Relative proportion (%)[a]		
	9-Ketone **1**	9,12-Cyclic hemiacetal **2**	6,9-Cyclic hemiacetal **3**
$CDCl_3$	95	3	2
C_5D_5N	94	3	3
$(CD_3)_2CO$	90	5	5
$(CD_3)_2SO$	83	13	4
CD_3OD	81	14	5
$(CD_3)_2NCDO$	80	15	5

[a] Measured from the integrated ^1H NMR spectra.



obtained from ^{13}C SIMPLE NMR.*[10] Thus, addition of small amounts of D_2O to a solution of erythromycin in $[^2H_6]$-DMSO resulted in a splitting (into doublets) of the major hemiacetal signals owing to C-6 and C-18, and the minor hemiacetal signal owing to C-12 (Fig. 2). The signs and magnitudes of these splittings proved conclusively that there was a free hydroxy group at position-6 in the major hemiacetal and at position-12 in the minor hemiacetal. The complete SIMPLE results are summarised in Table 3, and were fully consistent with the proposed structures for the tautomers and the earlier spectral assignments.

Experiments similar to those in $[^2H_6]$DMSO showed that in $[^2H_7]$DMF and CD_3OD† the same three species were present in about the same proportions. Likewise, detailed examination of the ^{13}C NMR spectra of erythromycin in $CDCl_3$, $[^2H_5]$pyridine, and $[^2H_6]$acetone revealed the presence of the ketone **1** and the hemiacetals **2** and **3**; the relative abundances of tautomers in these solvents being **1** ≫ **2** ≥ **3** (Table 1). Some diagnostic ^{13}C chemical shifts are presented in Table 4.

Some further observations from the SIMPLE experiments are worth noting here. Firstly, the magnitude of the splitting on

* The SIMPLE (secondary isotope multiplet NMR spectroscopy of partially labelled entities) experiment involves the measurement of a proton-decoupled ^{13}C NMR spectrum for a sample in which groups containing exchangeable protons have been partially deuteriated. Carbons in the vicinity of exchangeable protons give multiple signals, owing to the deuterium isotope effect (*e.g.* observation of ^{13}C-OH plus ^{13}C-OD), provided that the rate of exchange is slow relative to the size of the isotope effect. In the absence of intramolecular hydrogen bonding or conformational equilibria, the effect is normally only observed for carbons two or three bonds removed from the exchangeable atom.

† Rapid hydrogen–deuterium exchange meant that the SIMPLE results using 1:1 CD_3OD–CH_3OH were less useful than those obtained with the other solvents in this study. The other NMR experiments were, however, just as conclusive in CD_3OD as in $[^2H_6]$DMSO and $[^2H_7]$-DMF.

Table 2 ^1H and ^{13}C NMR chemical shifts (δ) for erythromycin A tautomers **1**, **2** and **3** in $(CD_3)_2SO$ at 27 °C

	9-Ketone **1**		9,12-Cyclic hemiacetal **2**		6,9-Cyclic hemiacetal **3**	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	174.7	—	176.4	—	177.1	—
2	44.3	2.76	45.9	2.63	44.7	2.68
3	78.8	4.01	78.6	4.11	77.4	3.90
4	39.0	1.88	39.5	2.16	*a*	*a*
5	82.5	3.48	84.0	3.40	82.1	3.55
6	73.3	—	74.1	—	83.5	—
7	38.3	1.71, 1.28	36.2	1.90, 1.08	*a*	*a*
8	39.5	2.85	36.3	2.09	*a*	*a*
9	217.7	—	107.5	—	110.0	—
10	42.3	2.88	47.9	2.03	*a*	*a*
11	68.7	3.77	84.3	3.74	70.4	4.03
12	74.6	—	84.2	—	74.3	—
13	75.7	5.09	78.8	4.79	79.3	4.73
14	21.1	1.81, 1.38	23.4	1.82, 1.72	*a*	*a*
15	10.5	0.76	11.1	0.76	*a*	*a*
16	15.6	1.10	15.3	1.07	*a*	*a*
17	9.1	1.03	9.7	1.01	*a*	*a*
18	26.7	1.28	25.4	1.14	29.1	1.49
19	18.1	1.04	19.4	0.94	*a*	*a*
20	11.2	1.05	15.2	1.05	*a*	*a*
21	17.3	1.02	26.1	1.34	*a*	1.02
1′	102.2	4.37	103.6	4.28	102.8	4.32
2′	70.7	3.02	70.4	3.07	70.4	3.08
3′	64.2	2.43	64.2	2.51	64.2	2.43
4′	30.0	1.58, 1.07	29.9	1.58, 1.10	*a*	*a*
5′	67.1	3.61	67.7	3.52	67.3	3.61
6′	21.2	1.08	21.0	1.12	*a*	*a*
7′, 8′	40.2	2.21	40.2	2.21	40.2	2.21
1″	95.7	4.74	96.5	4.63	95.1	4.78
2″	34.9	2.28, 1.51	34.7	2.27, 1.50	*a*	*a*
3″	72.5	—	72.5	—	72.6	—
4″	77.4	2.89	77.4	2.89	77.4	2.89
5″	64.8	4.03	64.8	4.08	64.6	4.05
6″	18.5	1.17	18.0	1.16	*a*	*a*
7″	20.8	1.13	20.8	1.13	*a*	*a*
8″	48.8	3.21	48.8	3.19	48.8	3.19
6-OH[b]	—	4.48	—	*a*	—	—
12-OH[b]	—	4.22	—	—	—	*a*

[a] Not identified. [b] Assigned from ^1H, ^1H COSY-45. Other hydroxy groups could not be assigned unambiguously.

C-18 for the ketone **1** is almost twice that for the hemiacetal **2**, implying that there is a difference in the H–O–C(6)–C(18) dihedral angles in **1** and **2**.[11] Secondly, for the hemiacetal **2**, positive (high-frequency) isotope effects are shown by C-1′ in [$^2H_6$]DMSO, [$^2H_7$]DMF, [$^2H_6$]acetone, and 1:1 CD_3OD–CH_3OH, and by C-1″ in [$^2H_7$]DMF and [$^2H_6$]acetone (Table 3). These were attributed to isotope effects on hydrogen bonding or conformational equilibria.*,[12] Lastly, heavy resolution enhancement of the split C-18 resonance for the ketone **1** in [$^2H_7$]DMF resulted in the detection of a further splitting (Δ = *ca.* 17 ppb) of these signals. The origin of this second splitting is not known; however, it was noted that for the ketone **1**, before adding D_2O, many of the ^{13}C signals were very broad (*e.g.* C-3, C-4, C-5, C-10, C-11, C-16 and C-18 in [$^2H_6$]-DMSO; and C-4, C-7 and C-16 in [$^2H_6$]acetone). These effects suggest the presence of a conformational equilibrium in the ketone **1**. One such equilibrium for **1** in $CDCl_3$ has previously been described.[5]

To summarise, the evidence so far indicates that erythromycin A exists in various solvents as a tautomeric mixture of the ketone **1** (the predominant species) and two hemiacetals **2** and **3**, and that the proportion of the 9,12-cyclic hemiacetal **2**, relative to **1**, increases in the more polar solvents.

Ketone–hemiacetal tautomerism has also been reported for various derivatives of erythromycin. For example, 11-*O*-acyl[13] and 11-*O*-methanesulphonyl[14] derivatives of erythromycin A exist in solution exclusively as the hemiacetals, and these were thought to be the 6,9-cyclic hemiacetals. In fact, a cursory examination of the ^{13}C NMR spectrum of one of these derivatives, 4″-*O*, 11-*O*-diacetylerythromycin A,[13] in $CDCl_3$ suggested, in the light of our studies on erythromycin, that this may be the 9,12-cyclic hemiacetal (C-14 δ_c at 24.1).† We therefore undertook a detailed NMR spectroscopic examination of this derivative using the techniques employed for erythromycin, including ^{13}C SIMPLE NMR spectroscopy. This led to complete assignments of the ^1H and ^{13}C NMR spectra, and proved conclusively that the compound was indeed the 9,12-cyclic hemiacetal; some of the diagnostic assignments are summarised in Tables 5 and 6. From this result, it seems likely that other 11-*O*-acyl[13] and 11-*O*-methanesulphonyl[14] derivatives of erythromycin A also exist in the 9,12-cyclic hemiacetal form. To complete the structural studies on the 4″,11-diacetate we used NOE experiments to investigate the stereochemistry at C-9. Experiments in $CDCl_3$, C_6D_6 and [$^2H_6$]DMSO proved inconclusive, but in [$^2H_5$]pyridine irradiation of 6-OH gave a definite and reproducible effect (*ca.* 3%) on 13-H. This result, coupled with model building, led us to propose the (9*S*) stereochemistry, and hence structure **4** for the 4″,11-diacetate. Furthermore, comparison of chemical shifts (C-9, C-18, C-21) suggested that erythromycin 9,12-cyclic hemiacetal **2** may also have the (9*S*) stereochemistry, *i.e* structure **5**.

In the above studies, as in those on erythromycin, some interesting effects were observed in the ^{13}C SIMPLE experiment. Positive (high-frequency) isotope effects were observed for C-3, C-1′,‡ and C-1″ (Table 6), and were ascribed to effects on conformational equilibria or hydrogen bonding. Also, for C-3, C-5, C-6 and C-1′ the resonances were shifted to higher field (Fig. 3) following the addition of D_2O (5 mm^3 in 500 mm^3 $CDCl_3$); these shifts were ascribed to changes in solvation or conformational equilibria. It was also noted that the resonances of C-5 and C-7 were markedly broadened, relative to those of the other non-quaternary carbons, prior to the addition of D_2O; this effect is also consistent with **4** being involved in a conformational equilibrium, the rate of which, for C-5 and C-7, is intermediate on the NMR timescale. On partial deuteriation, the C-9 and C-2′ signals broaden, but show no resolved splitting,‡ whereas the C-6 and C-18 signals split but remain sharp; this indicates that exchange at the 6-OH is slow compared with that at the other hydroxy groups, and implies intramolecular hydrogen bonding of the 6-hydroxy.[11]

Taken together, one interpretation of the above effects is that they result from conformational equilibria being perturbed by deuteriation of the 6-hydroxy group and the effect

* This must be true for C-1″, since this carbon is five bonds removed from the nearest exchangeable hydrogen. It must also be true for C-1′ in [$^2H_6$]acetone since C-2′ shows no discernible splitting, and therefore is probably also true for C-1′ in the other solvents.

† In this context it is worth mentioning that the 11-*O*-acetyl and 11-*O*-methanesulphonyl derivatives of erythromycin B, which lack the 12-hydroxy group, exist in solution exclusively as the 9-ketones.[13,14]

‡ The absence of a resolved splitting of the C-2′ signal clearly indicates that the isotope effect at C-1′ is not a direct three-bond effect resulting from exchange at the 2′-OH.

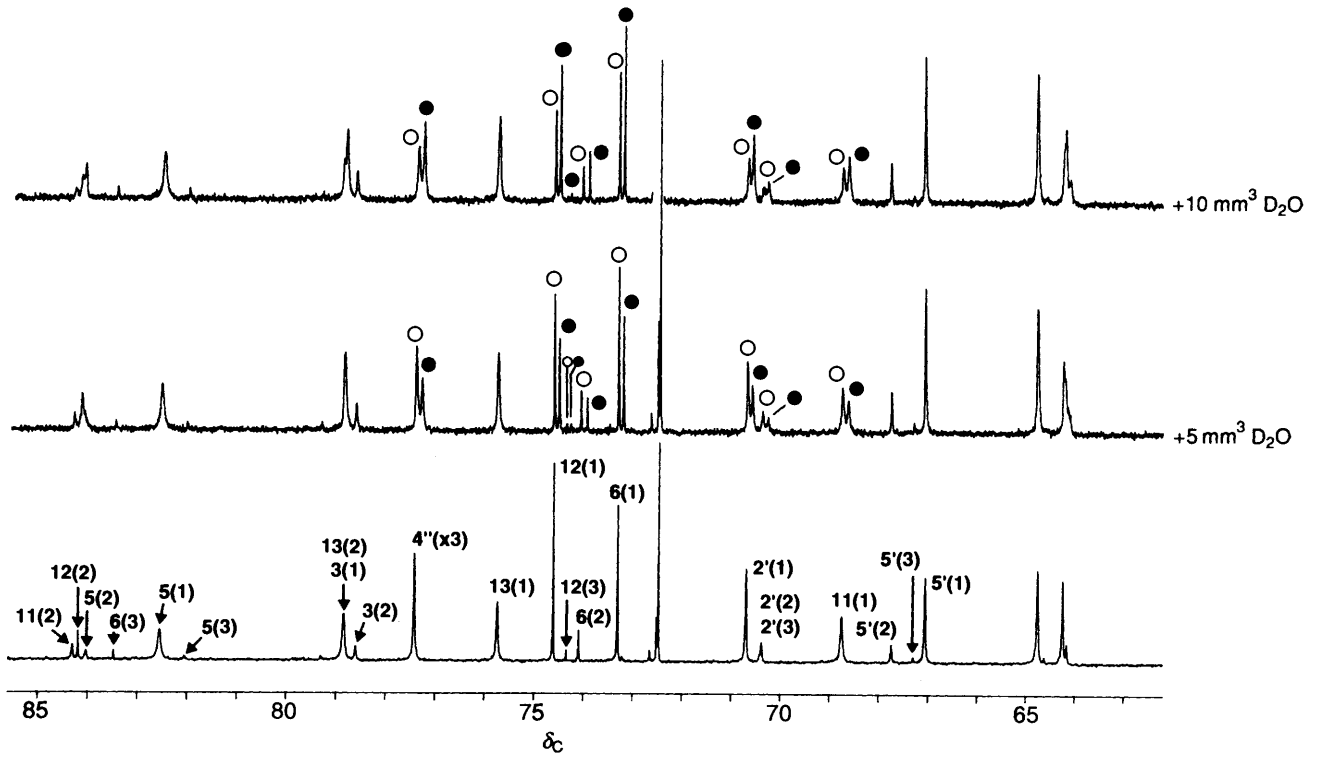


Fig. 2 Part of the ^{13}C NMR spectrum of erythromycin A in $(CD_3)_2SO$–Me_4Si, and after the addition of 5 and 10 mm³ D_2O. Resonances for tautomers **1**, **2** and **3** are indicated. Filled and unfilled circles indicate resonances in deuteriated and protiated isotopomers, respectively.

Table 3 Isotope effects (Δ in ppb), in the ^{13}C NMR spectra, on partial deuteriation of hydroxy groups of erythromycin A 9-ketone **1**, 9,12-cyclic hemiacetal **2**, and 6,9-cyclic hemiacetal **3** in $(CD_3)_2SO$, $(CD_3)_2NCDO$ and $(CD_3)_2CO$ at 27 °C[a]

	Solvent								
	$(CD_3)_2SO$			$(CD_3)_2NCDO$			$(CD_3)_2CO$[b]		
Carbon	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
6	−102	−127		−109	−128		−120	*ca.* −135	
9		−89	−89		−83	−94			
10							−26		
11	−114	−119	*d*	−102			*ca.* −71[g]		
12	−102		−80	−103		*ca.* −85	−108[g]		
18	−80	−42		−78[f]	−38		−88	*ca.* −40	
20	−13		*e*			*e*			*e*
21	−51		*e*			*e*	−40[g]		*e*
1′		+13			+21			*ca.* +25	
2′	−102	−101	*d*	−95	−103		*c*	*c*	
3′	*c*								
1″					+16			*ca.* +19	
4″	−127		*d*	−105			*c*		

[a] In 1:1 CD_3OD–CH_3OH positive isotope effects were observed for C-3 (*ca.* +25) and C-1′ (*ca.* +25). [b] Chemical shifts assigned by comparison with spectra in $[^2H_6]$DMSO, $[^2H_7]$DMF, and $CDCl_3$. [c] Broadening of signal, but no measurable splitting. [d] Not observed owing to overlapping resonances. [e] Resonance not identified. [f] Split further (Δ *ca.* 17 ppb) on heavy resolution enhancement. [g] Broad.

that this has on its hydrogen bonding. Other explanations may be possible.*

Two other derivatives of erythromycin for which ketone–hemiacetal tautomerism is well documented are the 11,12-cyclic carbonate[15] **6** and the 11,12-cyclic methylene acetal[16] **7**. ^{13}C NMR spectroscopic studies have shown that both these compounds exist in $CDCl_3$ as mixtures of the 9-ketone (minor component) and two 6,9-cyclic hemiacetals. Furthermore, for the cyclic carbonate, one of the hemiacetals has been isolated by crystallisation and shown to have the (9*R*) stereochemistry **8** by X-ray crystallography.[17] From these literature data[15,17] and our own NMR spectroscopic studies we recognised that certain ^{13}C chemical shifts (particularly C-7, C-9 and C-18) were diagnostic for the stereochemistry at C-9 in the hemiacetals **8**–**11**, and this proved useful in allowing us to draw conclusions regarding the stereochemistry at C-9 in erythromycin 6,9-cyclic

* One such explanation, that the positive isotope effects result from perturbation of conformational equilibria involving the sugar rings, seemed to us less likely. Non-selective ^{13}C relaxation time measurements showed that, as for **1**,[5] the desosamine and cladinose moieties have little motional freedom relative to the aglycone, with average ^{13}C NT_1s of 0.31 ± 0.01, 0.30 ± 0.02 and 0.27 ± 0.02 s, respectively (N = no. of protons on methylene and methine carbons; T_1 = spin-lattice relaxation time; error limits are ranges).

Table 4 Some diagnostic ^1H and ^{13}C NMR chemical shifts (δ) for erythromycin A tautomers **1**, **2** and **3**

	δ[a]		
Atom	9-Ketone **1**	9,12-Cyclic hemiacetal **2**	6,9-Cyclic hemiacetal **3**
C-6	74	74	85
C-9	219	108	111
C-12	75	85	75
C-14	22	25	22
C-18	27	26	30
C-21	18	27	*b*
18-H	1.4	1.2	1.6
21-H	1.1	1.4	1.0

[a] Averaged chemical shift from spectra in $(CD_3)_2SO$, CD_3OD, and $(CD_3)_2NCDO$ at 27 °C. [b] Not identified.

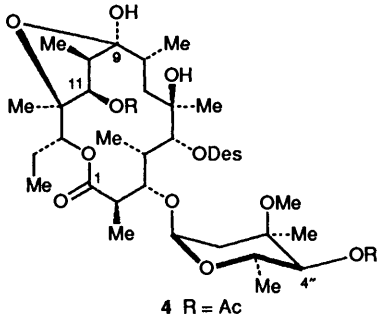
Table 5 Some diagnostic ^1H and ^{13}C NMR chemical shifts (δ) for compound **4** in $CDCl_3$–Me_4Si at 27 °C

Atom	δ
C-6	74.7
C-9	108.6
C-12	84.8
C-14	24.1
C-18	25.5
C-21	25.2
18-H	1.26
21-H	1.51

Table 6 Isotope effects (Δ in ppb), in the ^{13}C NMR spectrum, on partial deuteriation of the hydroxy groups in compound **4** in $CDCl_3$–Me_4Si at 27 °C

Position	Δ
3	+25
5	*a*
6	−130
9	*a*
11	0
12	0
18	−42
21	0
1′	+17
1″	+11

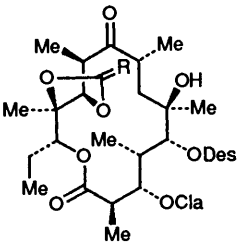
[a] Resonance broadened, but no measurable splitting.



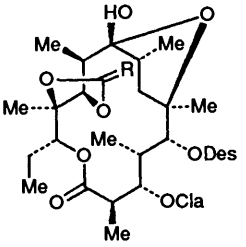
4 R = Ac
5 R = H

hemiacetal **3**. The relevant shifts are presented in Table 7, and led us to conclude that compound **3** probably has the (9*S*) stereostructure **12**.

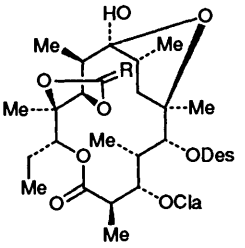
For completion, a number of other derivatives which exhibit



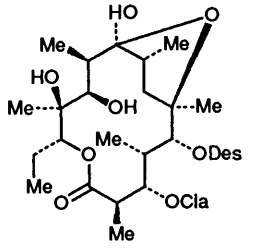
6 R = O
7 R = H_2



8 R = O
9 R = H_2



10 R = O
11 R = H_2



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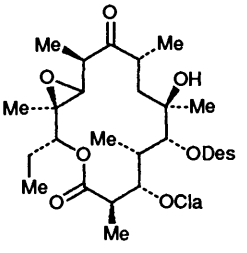

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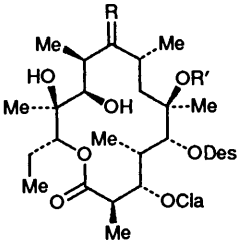

14 R = NH, R′ = H
15 R = O, R′ = Me
16 R = NH, R′ = Me

ketone–hemiacetal tautomerism are worth mentioning. The 11,12-epoxide **13** is present in solution as the ketone **13** and one 6,9-cyclic hemiacetal, and the proportion of tautomers was found to vary with the temperature and the solvent.[14] (8*S*)-8-Hydroxyerythromycin A[18] and (8*S*)-8-fluoroerythromycin A[19] exist in solution as the ketone and one 6,9-cyclic hemiacetal. 11-*O*-Alkyl derivatives of erythromycin A[20] are present in $CDCl_3$ as mixtures of the ketone and one or two hemiacetals.* Similar ring–chain tautomerism has also been observed for erythromycin 9-imine **14**, which in $CDCl_3$ exists as a mixture of the imine **14** and two α-amino cyclic ethers.[23]

The factors governing tautomerism in erythromycin and its derivatives are by no means clear, but from our own studies and those in the literature it is possible to draw some general conclusions. There appears to be ample evidence from solution-state IR carbonyl stretching frequencies[13] and C-9 chemical

* Ref. 20*b* describes the 11-methyl, ethyl, and propyl ethers of erythromycin A as being 1:1 mixtures of ketone and 6,9-cyclic hemiacetal in $CDCl_3$; studies in these laboratories with the 11-ethyl ether suggest a *ca.* 1:1 mixture of ketone and 9,12-cyclic hemiacetal.[21] 11-Alkyloxymethyl ethers,[20a] on the other hand, exist in $CDCl_3$ as three tautomers: the ketone, a 6,9-cyclic hemiacetal, and a 9,12-cyclic hemiacetal (major species).[22]

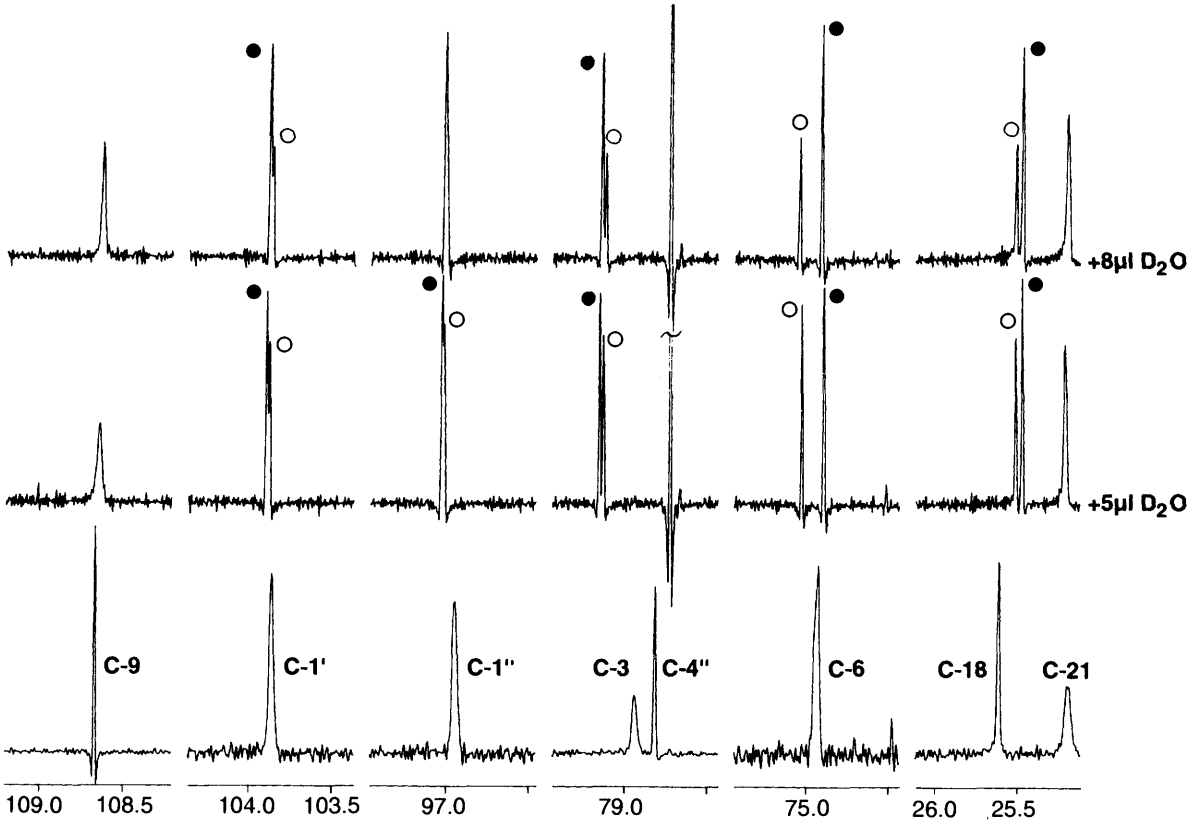

Fig. 3 A selection of resolution enhanced ^{13}C resonances for compound **4** in $CDCl_3$–Me_4Si, and after addition of 5 and 8 mm^3 D_2O. Filled and unfilled circles indicate resonances in deuteriated and protiated isotopomers, respectively.

Table 7 C-7, C-9 and C-18 chemical shifts (δ_C) for compounds **3** and **8–11** in $CDCl_3$–Me_4Si at 27 °C

	3	**8**	**9**	**10**	**11**
δ_{C-7}	*a*	39.6	38.8	43.3	43.0
δ_{C-9}	110.5	107.2	107.6	109.9	111.3
δ_{C-18}	29.8	26.4	26.8	31.8	31.5

[a] Not identified.

shifts[24] for hydrogen bonding between the 11-hydroxy and 9-carbonyl in erythromycin A. This hydrogen bonding will have a stabilising influence on the 9-ketone; and therefore, provided that there is no comparable stabilising effect in the hemiacetals, derivatisation of the 11-hydroxy would be expected to destabilise the ketone relative to the hemiacetals. This appears to be the case. Thus, the 11,12-cyclic derivatives **6**, **7** and **13** favour the 6,9-cyclic hemiacetal form in solution, and 11-*O*-acyl and 11-*O*-alkyl derivatives favour the 9,12-cyclic hemiacetal form. Hydrogen bonding involving the 11-hydroxy could also be a factor in determining the relative stabilities of ketone and hemiacetal forms of erythromycin in various solvents. In the hemiacetals, hydrogen bonding between the more polar solvents and the 11-hydroxy would increase the stability of these forms relative to the ketone (in which the 11-hydroxy is intramolecularly hydrogen bonded to the 9-carbonyl). Thus the proportion of hemiacetals increases in the more polar, hydrogen bonding solvents. One further point is worth making regarding possible stabilising effects in the 9,12-cyclic hemiacetal. The ^{13}C NMR spectra of 6-*O*-methylerythromycin A[24] **15** in $CDCl_3$ and CD_3OD show signals for the ketone **15** only; there is no evidence for hemiacetal forms in these solvents. Similarly, in $CDCl_3$, only the ketone form appears to be present for the 6,11-dimethyl ether of erythromycin A,[24] and the 6-methyl ether of erythromycin A imine shows absorptions for only the imine **16**.[23] Since, in principle, all these compounds could form a 9,12-cyclic tautomer, the implication is that stabilisation of this form requires a free hydroxy at position -6.* Evidence from ^{13}C SIMPLE NMR spectroscopy for hydrogen bonding involving the 6-hydroxy in the 9,12-cyclic hemiacetals of erythromycin and its 4″,11-diacetate was noted earlier, and provides some support for this hypothesis.

Conclusions.—Erythromycin A exists in solution as a tautomeric mixture of the ketone **1** and the cyclic hemiacetals **2** and **3**; the (9*S*) stereochemistry is provisionally assigned to these hemiacetals, *i.e.* structures **5** and **12**. Similar tautomeric forms have previously been reported in the literature for various derivatives of erythromycin. It is proposed that hydrogen bonding involving the 6- and 11-hydroxy groups is important in determining the stabilities, and hence the relative proportions, of the various tautomeric forms in solution.

Following the earlier work on the conformation of the ketone **1**,[5] the current studies give us a more complete picture of the nature of erythromycin A in solution. This knowledge, we believe, should help towards a better understanding of the chemistry of erythromycin and its derivatives, and, as pointed out by Barber and co-workers,[25] should contribute to a proper understanding of the antimicrobial activity of the 14-membered macrolides at the molecular level.

Experimental

Materials.—Erythromycin A was purchased from K and K-Greef Chemicals Ltd. and was crystallised from $CHCl_3$–hexane several times to give material which was homogeneous (>98%) by HPLC. Erythromycin A 4″,11-diacetate was prepared by the

* The alternative, namely that methylation of the 6-hydroxy provides extra stabilisation of the ketone (imine) form, seems to us less likely.

method of Jones *et al.*,[13] and was purified by chromatography (silica gel; 1:9:90 35% aq. NH_3–MeOH–CH_2Cl_2), to give compound **4** as a white foam.

NMR spectroscopy.—All ^1H and ^{13}C NMR spectroscopic experiments were conducted using standard pulse sequences on a Bruker AM 400 NMR spectrometer fitted with a 5 mm $^1H/^{13}C$ dual probe. A sample concentration of 70–100 mg cm^{-3} was used, with Me_4Si as an internal standard. *J* values are given in Hz. Experiments were run at 27 °C.

1D NMR experiments included normal and resolution enhanced ^1H NMR spectra, and DEPT and broad-band decoupled ^{13}C NMR spectra. The ^{13}C SIMPLE experiments were conducted by adding 5mm^3 aliquots of D_2O to the solutions and recording the spectra using broad-band decoupling and DEPT pulse sequences after each addition.

The 2D ^1H, ^1H COSY-45 spectra were acquired at 400 MHz with sweep widths of 2000 Hz into 2048 data points in F2. The relaxation delay was 1s and each F.I.D. (free induction decay) was acquired with between 32 and 64 scans and 4 dummy scans. 512 Values of the evolution time were sampled, but the data were zero-filled to 1024 points in F1 prior to double Fourier transformation with unshifted sine-bell functions in both dimensions. 2D ^1H, ^{13}C COSY spectra were acquired with sweep widths of 8000 Hz into 2048 data points in F2 and 2000 Hz into 128 data points in F1. F.I.D.s were acquired with between 160 and 320 scans with a relaxation delay of 1s. 2D ^1H, ^{13}C COLOC spectra were acquired with sweep widths of 17–20 kHz into 8192 data points in F2; 256 evolution increments were used. A polarisation delay time for long range coupling of 54 ms and a refocussing delay of 27 ms were implemented, as was a relaxation delay of 1s. The data were zero-filled to an 8K × 1K matrix prior to double Fourier transformation.

In the ^1H NOE difference spectroscopy experiments, 4–8 irradiations were performed in one experiment with automatic cycling through the frequency list, using 16 scans and 4 dummy scans at each frequency. A pre-irradiation delay of 3s was used, followed by a sub-saturation irradiation period of 3s. A 2 Hz line broadening function was applied to each F.I.D. prior to Fourier transformation. The total number of scans for each spectrum was in the region of 800–1000.

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