Isomerization of Erythromycin A in Deuterium Oxide and [$^2H_6$] Dimethyl Sulphoxide Solutions: a ^1H and ^{13}C NMR Study

Jill Barber,*[a] Jeffrey I. Gyi,[a] Gareth A. Morris,*[b] David A. Pye,[a] and James K. Sutherland[b]

Departments of [a]Pharmacy and [b]Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK

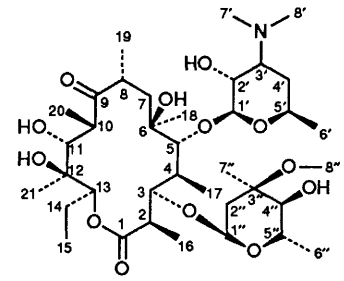
Erythromycin A exists as an equilibrium mixture of two isomers in solvents such as water and dimethyl sulphoxide; ^1H and ^{13}C NMR spectroscopy has been used to elucidate the two structures.

Erythromycin A is a clinically important macrolide antibiotic which acts by binding to bacterial ribosomes, so inhibiting protein biosynthesis.[1] The crystal structure has been determined,[2,3] and full assignments of the ^1H and ^{13}C NMR spectra in $CDCl_3$ solution have been published.[4] The structure of the drug in the physiologically more relevant medium of aqueous solution has, however, attracted little attention. Full ^1H and ^{13}C NMR assignments in aqueous solution were required for NMR studies of binding to ribosomal components, and so spectra were examined in aqueous buffer (50 mM sodium phosphate, 200 mM KCl in D_2O; apparent pH 7.4). Proton spectra show immediately that in aqueous buffer erythromycin A exists as a mixture of two components in a ratio of 2.5 : 1 at room temperature. COSY, HOHAHA (homonuclear Hartmann Hahn) and ^1H–^{13}C shift correlation experiments at 500 MHz were used to confirm that the major component is the ketone (**1**) found in the crystal and in $CDCl_3$ solution. Complete ^1H and ^{13}C assignments were obtained for (**1**), with the exception of the three quaternary carbons C-6, C-12 and C-3″ around δ 75.

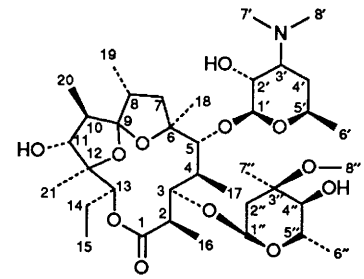
The ^{13}C spectrum for the minor component shows no apparent ketone signal, but a quaternary carbon signal at δ 109, suggesting the formation of an enol or hemiketal. Investigations were complicated by the low solubility of the drug (15 mg total erythromycin A ml^{-1}) and by spectral overcrowding. Partial assignments were possible, the presence of signals H-8 and H-10 showing that the minor component is not an enol. Formation of cyclic hemiketals is observed in a number of other macrolide antibiotics, and the existence of such derivatives as anhydroerythromycin A (**2**) suggests that the minor component might be a 6–9 or 12–9 hemiketal such as (**3**) or (**4**). Exchange between the components is slow on the NMR timescale at all temperatures used, but the ratio between the components varies from 1 : 1 at 6 °C to 5 : 1 at 50 °C, implying a large enthalpy difference between the two components.

Although most of the minor component assignments in aqueous buffer were eventually completed, efforts to identify the crucial signals C-18, C-6, H_3-18, C-21, C-12, and H_3-21, and hence determine the mode of cyclisation, were unsuccessful in all the protic solvents used. In the majority of aprotic solvents the drug exists almost completely as the ketone, but in dimethyl sulphoxide (DMSO) the major : minor ratio is about 5 : 1 at room temperature; happily, the drug is also extremely soluble in this solvent (500 mg ml^{-1}). Full assignments were made at 300 MHz in [$^2H_6$]DMSO for both components, using COSY, directly detected ^1H–^{13}C shift correlation, NOESY (negative Overhauser effects were observed), and the heteronuclear multiple bond correlation (HMBC) experiment;[5] the latter spectrum (see Figure 1) allowed assignment of the crucial quaternary carbons. A number of interesting effects were observed, including evidence in the COSY spectrum for long range couplings in the major component between OH-6 and H_3-18, and between OH-12 and H_3-21.

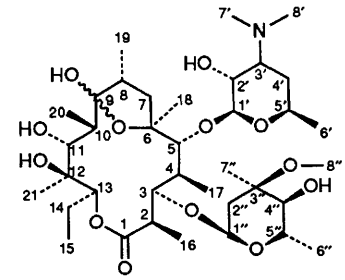
The assignments obtained for the minor component were entirely consistent with hemiketal formation, with chemical



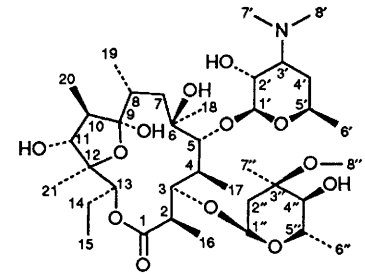
(1)



(2)



(3)



(4)

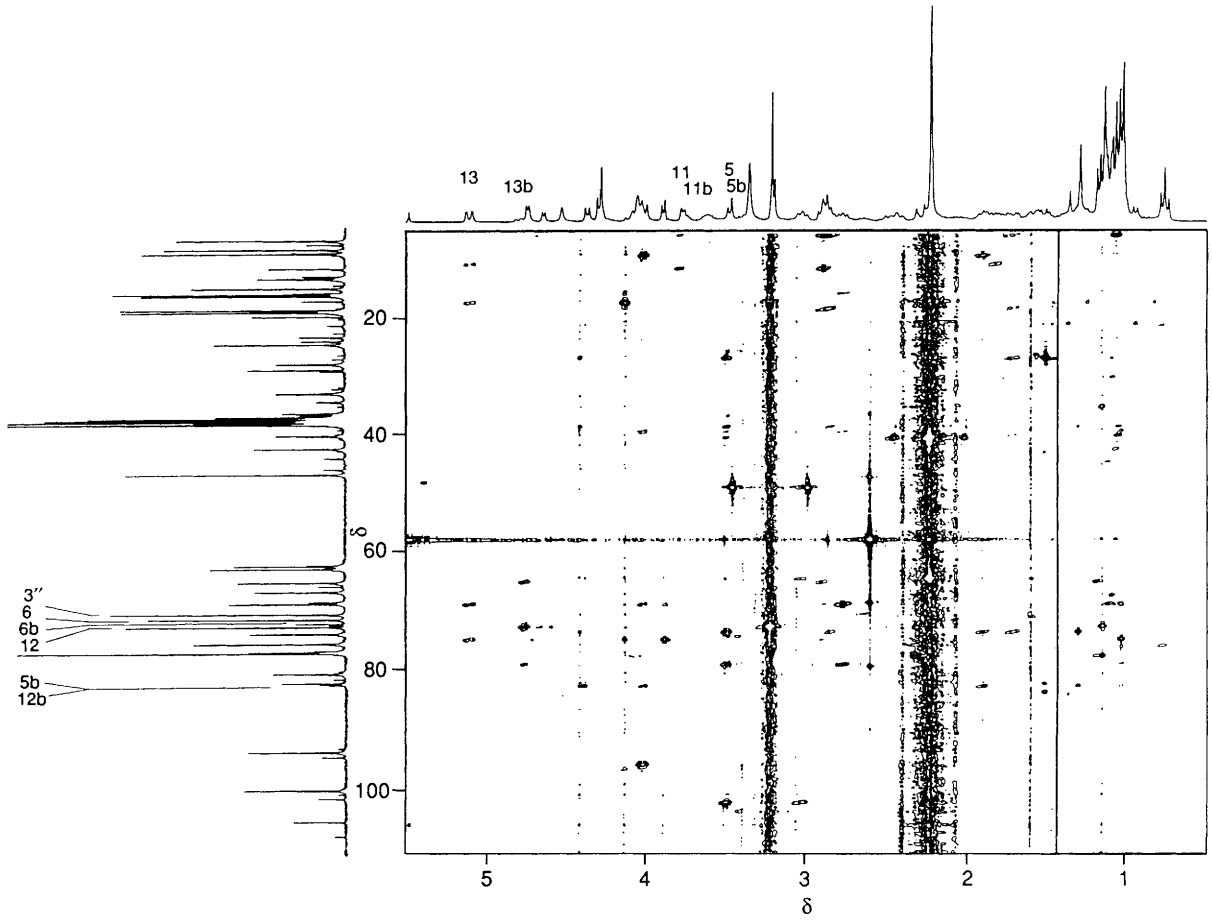


Figure 1. HMBC spectrum of erythromycin A in [$^2H_6$] DMSO, with ^1H and ^{13}C 1D spectra. The vertical scale of the 2D spectrum has been decreased eight-fold for the methyl region, f_2 = 0.5—1.4 ppm. Note the doubling of signals in the 1D ^1H spectrum, *e.g.*, MeO 8″ at δ 3.2. Important assignments for the major and minor (b) isomers are marked on the 1D spectra.

shifts suggestive of 12–9 cyclisation; definitive proof was obtained by measuring a decoupled ^{13}C spectrum of a [$^2H_6$]-DMSO solution to which one drop of D_2O had been added. This led, with the small amount of H_2O already present in the sample, to approximately equal concentrations of H_2O and D_2O, and hence to the observation of ^{13}C doublets (split by the secondary isotope effect) for all carbons bearing OH groups.[6] Doublets were seen for C-6 and C-12 in the major isomer, but only for C-6 in the minor isomer. It is concluded that the minor isomer of erythromycin A in DMSO solution is the 12–9 hemiketal. Dilution experiments with mixed solvents were used to confirm that the minor isomer in DMSO solution is the same as that in aqueous buffer. Molecular modelling studies suggest that *R* stereochemistry at C-9 (*i.e.*, a *cis* ring junction) is likely to be preferred (8 kcal mol^{-1}) over *S*, giving the structure (**4**).

It has been proposed that cyclic hemiketal derivatives of erythromycin A may be able to induce resistance to the drug by promoting the methylation of 23*S* ribosomal RNA.[7] The structure elucidation presented here should facilitate experimental and modelling studies of the biological activity of the two components of erythromycin A expected *in vivo*.

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