Novel Conformations of Erythromycin Antibiotics: the Conformational Analysis of Erythromycin A and (9*S*)-9-Hydroxy-9-deoxoerythromycin A by N.M.R. Spectroscopy

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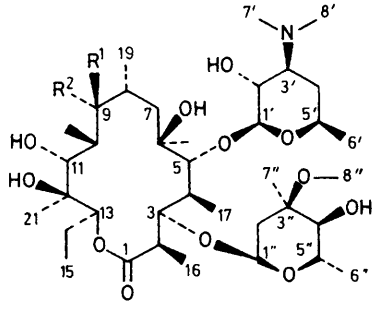
^{1}H and ^{13}C N.m.r. spectroscopy combined with molecular modelling has been used to characterise novel 'folded-in' lactone ring conformations of erythromycin A (**1**) and its (9*S*)-9-hydroxy-9-deoxo analogue (**2**) in $CDCl_3$ solution.

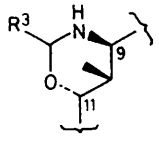
Erythromycin A (**1**) is a medically important macrolide antibiotic composed of a poly-functionalised 14-membered lactone ring substituted with desosamine and cladinose sugar units. In view of their medical importance, the solution conformations of various erythromycins and their aglycones have been studied by many techniques including n.m.r. spectroscopy.[1,2] The previous n.m.r. work was hampered by incomplete and/or incorrect assignments and by the reliance on vicinal proton–proton coupling constants to determine conformations. Unambiguous assignments for the ^{1}H and ^{13}C n.m.r. spectra of (**1**)[3] and (**2**)[4,5] have recently been established, thus enabling the more detailed conformational studies described here to be carried out.[6] Extensive 400 MHz ^{1}H nuclear Overhauser enhancement (n.O.e.) difference experiments on (**1**) in $CDCl_3$ solution led to the observation of 122 n.O.e.s, including intra-lactone, lactone–sugar, inter-sugar, and intra-sugar effects. Of these 122 n.O.e.s, 114 corresponded to interactions between protons $\leq$3 Å apart in the crystal structure[7] of the hydroiodide dihydrate of (**1**), conformation (**A**) (Figure 1a). Of the eight outstanding n.O.e.s, five were small *trans*-diaxial interactions and one was a small n.O.e. from CH_3-17 to H-5, *i.e.* n.O.e.[17]5, all of which were compatible with conformation (**A**) ($r_{H,H}$ <3.3 Å). The two remaining n.O.e.s were small effects between H-3 and H-11.

A typical ^{1}H n.O.e. difference spectrum for (**1**), obtained on irradiation of H-11, is shown in Figure 2(a). N.O.e.s to H-4, H-7ax, H-10, H-13, and CH_3-21 were clearly observed and were all compatible with the crystalline-state conformation (**A**), *i.e.* $r_{H,H}$ <3 Å in (**A**). However the small (~0.2%) n.O.e.[11]3 was unexpected [$r_{3,11}$ ~3.7 Å in (**A**)]. Although small, the rate of build-up of n.O.e.[11]3 was no slower than n.O.e.[11]4 [$r_{11,4}$ ~2.2 Å in (**A**)]. Variable solvent and variable temperature ^{1}H n.m.r. experiments indicated that in CD_3OD, $(CD_3)_2SO$, [$^2H_5$]pyridine, and [$^2H_7$]dimethylformamide (DMF) the C-6 to C-9 region of (**1**) became conformationally unstable. In $(CD_3)_2SO$ especially, the low value of $^3J_{2,3}$ (~8.5 Hz at 340 K) also indicated fast conformational averaging around the C-2–C-3 bond. It was concluded that the two unexpected n.O.e.s between H-3 and H-11 were probably due to a very minor 'folded-in' lactone ring conformation ($r_{3,11}$ <3 Å) of (**1**) in fast equilibrium with a major conformation. The major conformation is very similar to (**A**) except that the orientation of the two sugar rings is slightly different.

The ^{1}H n.O.e. difference experiments were repeated on (**2**).[4] Whilst (**2**) exhibited all but three of the intra-lactone n.O.e.s of (**1**), eleven *new* intra-lactone n.O.e.s were found not including those involving H-9. Irradiation of H-11 (Figure 2b) now gave an n.O.e.[11]8, unobserved in (**1**), and n.O.e.[11]3 was larger than n.O.e.[11]4. The lactone ring $^3J_{H,H}$ values of (**2**)[5] were different from those of (**1**)[3] and further large changes were observed when the $^3J_{H,H}$ values were measured in CD_3OD. On the basis of this and other n.m.r. evidence it was concluded that (**2**) existed in solution as a mixture of lactone ring conformations in fast exchange. The



(**1**) R^1, R^2 = O
(**2**) R^1 = OH, R^2 = H



(**3**) R^3 = $CH_2OCH_2CH_2OMe$

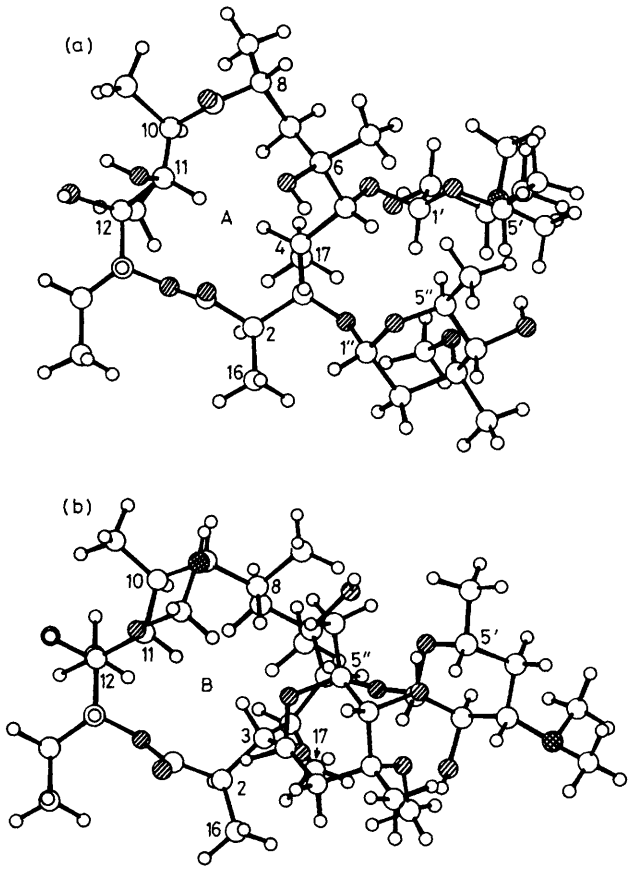


Figure 1. (*a*) The crystal structure[7] (**A**) of erythromycin A hydroidide dihydrate, shown without the water molecules or HI. (*b*) the crystal structure[8] (**B**) of the erythromycylamine A derivative (**3**) shown without the ether side-chain for clarity.

lactone ring conformations were characterised as belonging to two types: 'folded-out,' *e.g.* (**A**), or 'folded-in' in the C-3 to C-5 region such that H-3 and H-11 were spatially proximate. For (**2**) each type of conformation is unstable in the C-6 to C-9 region but stable in the C-10 to C-13 region and each type is significantly populated. A crystalline-state model for the 'folded-in' conformation was found in the crystal structure[8] of the erythromycylamine derivative (**3**) (Figure 1b). When 'destabilised' in the C-6 to C-9 region this 'folded-in' conformation (**B**) displayed all the new, close proton–proton interactions demanded by the n.O.e. data on (**2**). The conformational conclusions for (**1**) and (**2**) are novel and contradict often-repeated statements[1,2] that in solution erythromycin antibiotics exist in a single, stable, alternate diamond-lattice conformation very similar to (**A**) [although the possibility of a conformational equilibrium in the C-6 to C-9 region of (**1**) has been noted[2]].

These novel conclusions were further tested using ^{13}C n.m.r. relaxation time experiments.[9] Conformation (**A**) is characterised by a very high calculated† energy barrier to the rotation of Me-16, due to the close approach of H-1″ (r_{min} ~1.29 Å). In (**B**) (Figure 1b), the inward folding of the lactone ring removes the steric hindrance to the rotation of Me-16 and the calculated energy barrier drops by a factor of ~5. In good agreement with the calculation for (**A**) the ^{13}C NT_1 value of Me-16 in (**1**) was very short (0.78 ± 0.04 s) relative to that of

† Calculations used the MODEL program in the CHEMGRAF program suite.[12]

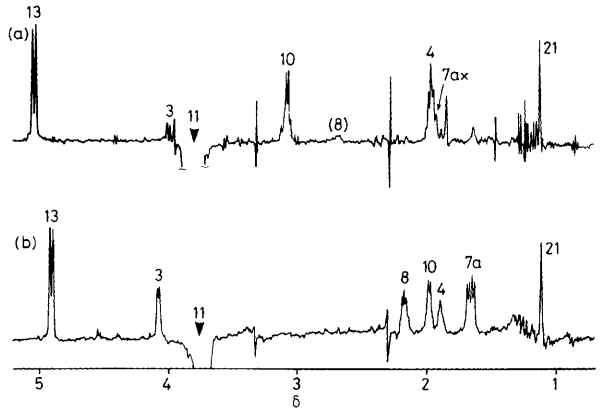


Figure 2. The 400 MHz n.O.e. difference spectra obtained by irradiation of H-11 in (a) (**1**), and (b) (**2**).

any other methyl group [*e.g.* Me-17 (1.80 s)] and indicative[10] of great hindrance to rotation. By contrast in (**2**), the ^{13}C NT_1 values of Me-16 (1.25 s) and Me-17 (1.23 s) were approximately equal and consistent with the population of conformations such as (**B**) [as well as (**A**)] with reduced energy barriers to the rotation of Me-16. The antibacterial activity of (**2**) against a range of representative organisms is in the range 16—32 times lower than that of (**1**).[11]

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