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THE CONFORMATION OF MACROLIDE ANTIBIOTICS II.

CONFIGURATIONAL AND CONFORMATIONAL STUDIES OF DIHYDROERYTHRONOLIDES

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Nuclear magnetic resonance techniques have recently been applied to the determination of the solution conformation of the 14-membered lactone in the erythromycin series (1,2), and the conformations revealed by these independent studies are in substantial agreement. One of the points of disagreement concerns the orientation of the C_{11}-OH. In the conformation proposed by Demarco (2), the quasi-equatorial orientation of C_{11}-OH is based on the application of aromatic solvent induced shifts (ASIS) (3) in the nmr spectra of some dihydroerythronolides. In the conformation proposed by Perun and Egan (1) the quasi-axial orientation of C_{11}-OH (see representation I or II) is similar to the orientation in the crystal structure (4), and is supported by additional nmr and chemical evidence presented in this communication.

Chemical evidence - The epimeric (9S) - and (9R)-9-dihydroerythronolide B compounds Ia and IIa were obtained by sodium borohydride reduction of erythronolide B in methanol, followed by chromatographic separation; (9S)- and (9R)-9-dihydro-6-deoxyerythronolides (Ib and IIb) were similarly prepared from 6-deoxyerythronolide (5). In both cases the 9S-epimer was the major product and had the greater chromatographic mobility on silica gel.

The configurations at C_9 were determined by chemical evidence and are based on a comparison with (9S)-9-dihydroerythronolide A (Ic), a compound with known configuration at C_9 (6,7). The three 9S compounds (Ia-Ic) react with benzeneboronic acid in refluxing acetone to give the bis-phenylboronate derivatives IIIa-IIIc. The two 9R compounds (IIa and IIb) give the mono-phenylboronate derivatives IVa and IVb under the same conditions. This selective reactivity of the 9S compounds forming cyclic esters at positions 9 and 11 distinguishes them from the 9R compounds and establishes their configurational identity at C_9.

The chemical reactivity of the C_9 and C_{11} hydroxyls in the 9S compounds provides evidence for the quasi-axial orientation of C_{11}-OH. In conformation I the two hydroxyls have a parallel relationship with each other. The unreactivity of these hydroxyls in the 9R compounds also is consistent with this conformation (II) as the hydroxyl orientations are now $\sim$ 90° apart. A quasi-equatorial orientation on the other hand would produce opposite

reactivities of these epimers. Detailed nmr analysis of the dihydroaglycones and their corresponding phenylboronate esters (8) revealed no changes in the coupling constants ascribable to conformational changes during esterification.*

Nmr evidence - The previous study by Demarco (2) used ASIS to help determine the conformation of dihydroerythronolides in solution. In our study, the application of this useful method to some additional dihydroerythronolides shows that some of the previous conclusions (2) were in error, and the additional data provides further support for the quasi-axial orientation of C_{11}-OH. The Table compares the ASIS values of the 9-dihydro-6-deoxyerythronolides Ib and IIb with the values of the 9-dihydroerythronolide A and B compounds.

The large ASIS value of C_9-H in (9R)-9-dihydroerythronolide B (IIa) is also observed with 9R-9-dihydro-6-deoxyerythronolide B (IIb), and thus cannot be a result of proximity of C_9-H and C_6-OH as stated earlier (2). This effect can best be explained by the proximate relationship of C_9-H and C_{11}-OH in conformation II where the hydroxyl on C_{11} has a quasi-axial orientation.

Further evidence for the quasi-axial orientation is obtained from high-dilution infrared studies of some 9S-dihydroerythronolide derivatives which show a strong intramolecular hydrogen-bond between C_9-OH and C_{11}-OH. The hydrogen-bonded C_{11}-OH resonance can also be observed in the nmr spectra of 9S-dihydroerythronolides (in acid-free $CDCl_3$ solution).

The larger ASIS value for C_{13}-H in compounds with the 9S configuration as compared with the 9R compounds was previously observed by Demarco and was used as major evidence for his proposed conformation (2). The presence of an intramolecular hydrogen-bond between C_9-OH and C_{11}-OH offers an alternate explanation for this effect which is consistent with our conformation, I. This hydrogen-bond in the 9S compounds should increase the strength of the pyridine complex with C_{11}-OH, and since the observed solvent shifts are time-averaged effects, any increase in complex stability will increase the population of this complex and thus increase the magnitude of the complex-induced shift.

Further support for this explanation was obtained from the ASIS values for (9S)-9-dihydro-11-acetylerythronolide B (Ia with C_{11}-OH acetylated) as shown in the Table. The formation of a pyridine complex with C_{11}-OH is no longer possible with this compound and consequently there is a large decrease in the C_{13}-H ASIS value. Other 9S derivatives substituted at C_{11} show a similar decrease of the C_{13}-H ASIS value, and it is apparent that the presence of a free hydroxyl at C_{11}-OH is responsible for a large portion of this effect.

Although the earlier communications (1,2) showed the lactone orientation different from that derived from X-ray crystal analysis (4) recent evidence has shown that it is the same as that in the crystal (L. A. Mitscher, et al., accompanying communication). In the conformational representation I (or II) the conformational model of Celmer (9) has been modified somewhat to accommodate the change in lactone orientation and to relieve steric interactions in this highly substituted ring.

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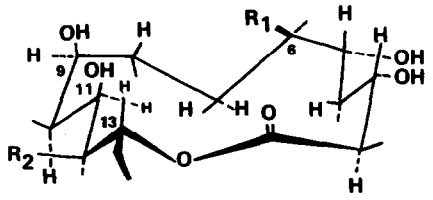
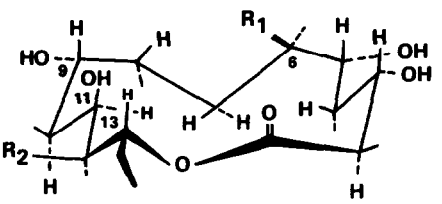
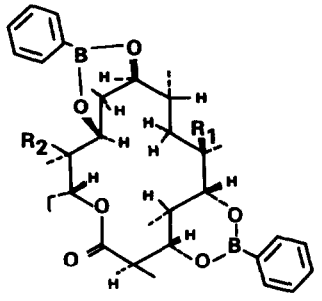
* Unequivocal spin-decoupling experiments performed on suitable derivatives (8) show that in the 9S compounds, $J_{8,9} \simeq 9.5$ Hz and $J_{9,10} \simeq 2.0$ Hz, correcting the values previously reported by Demarco (2). Similarly $J_{12,13}$ was confirmed to be the small value ($\simeq 1.0$ Hz) reported earlier (1) and not the alternate value (2).



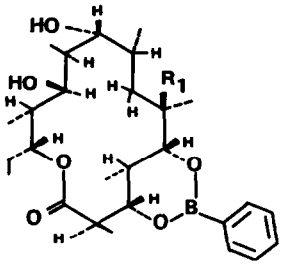
	R_1	R_2
Ia	OH	H
Ib	H	H
Ic	OH	OH



	R_1	R_2
IIa	OH	H
IIb	H	H



	R_1	R_2
IIIa	OH	H
IIIb	H	H
IIIc	OH	OH



	R_1
IVa	OH
IVb	H

TABLE

Aromatic Solvent Induced Shifts ($\delta CDCl_3 - \delta C_5D_5N$)

		H-2	H-3	H-5	H-9	H-11	H-13
9S-Dihydroerythronolide B	Ia	-0.26	-0.37	-0.43	-0.38	-0.67	-0.76
9S-Dihydro-6-deoxyerythronolide B	Ib	-0.26	-0.34	-0.26	-0.26	-0.51	-0.71
9S-Dihydroerythronolide A	Ic	-0.28	-0.40	-0.47	-0.37	-0.62	-1.02*
9S-Dihydro-11-acetylerythronolide B		-0.25	-0.36	-0.39	-0.41	-0.24	-0.38
9R-Dihydroerythronolide B	IIa	-0.27	-0.40	-0.50	-0.62	-0.44	-0.53
9R-Dihydro-6-deoxyerythronolide B	IIb	-0.29	-0.35	-0.29	-0.53	-0.37	-0.51

* Demarco (2) reports - 0.80 for this value by subtracting an amount corresponding to the contribution of C_{12}-OH (3).

The orientation of the lactone carbonyl cis to the C_{13}-H appeared to offer a further explanation for the large C_{13}-H ASIS value, since it has recently been shown that protons cis-related (1,3-diaxial) to the carbonyl of an ester or lactone experience a substantial negative benzene-induced solvent shift (10). We found to our surprise that the C_{13}-H ASIS value of all soluble dihydroerythronolides in benzene was negligible. In fact, the benzene-induced solvent shift of all ring protons was very small compared with the pyridine-induced effect. This indicates that with these compounds in pyridine the solute-solvent association must be a hydrogen-bond type of complex between the pyridine nitrogen and the hydroxyl proton (3). In support of this, 2,6-lutidine induces a much smaller solvent shift than pyridine, presumably because of its more hindered nitrogen.

Examination of a model of this modified conformation reveals that the plane of the macrolide ring divides the molecule into a hydrophilic portion and a hydrophobic portion. This appears to have some significance in the mechanism by which erythromycin inhibits protein synthesis (11).

Note: After submission of this manuscript a more detailed paper by Demarco appeared (12) which discusses some of the points of difference between the two proposed conformations. This paper presents a slightly modified conformation which is based upon our confirmed $J_{12,13}$ value (1.0 Hz). In this modification, however, the C_{11}-OH remains in a quasi-equatorial orientation, and in this orientation the relationship of protons on C_{11} and C_{12} is not consistent with the observed $J_{11,12}$ value (1,2).

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