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PYRIDINE SOLVENT SHIFTS IN THE NMR ANALYSIS OF ERYTHROMYCIN AGLYCONES

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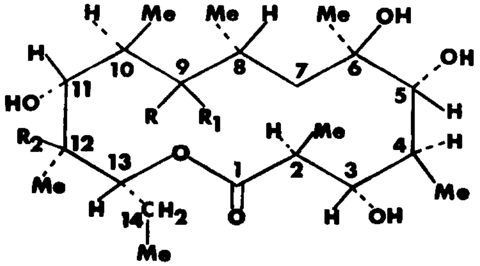
In previous NMR studies of solvent shifts induced by pyridine in compounds containing the hydroxyl function, certain solvent shift-structure relationships were derived (1,2) which were shown to be extremely useful in identifying salient structure features in the vicinity of solute hydroxyl functions. Consequently, the measurement of pyridine Δ-values ($\Delta = \delta CDCl_3 - \delta C_5D_5N$) provides a potentially powerful method for investigating configurational and conformational uncertainties in molecular systems containing the hydroxyl function.

We now wish to report the application of pyridine solvent shifts to the configurational and conformational analysis of several erythromycin aglycones, i.e. to the analysis of the two C-9 epimers of 9-dihydroerythronolide B, compounds 1 (3) and 2 (5), and 9-dihydroerythronolide A, compound 3 (5).



1 R=OH, R_1=H, R_2=H
2 R=H, R_1=OH, R_2=H
3 R=H, R_1=OH, R_2=OH

At the time this study was initiated, relative and absolute configurational assignments for the C-9 hydroxyl function in 1, 2, and 3 were as yet unproven, although for 3, previous studies (7,8) tentatively suggested, based on not entirely unambiguous results, that the configuration at C-9 was S. In addition, recent X-ray studies (8) have defined the conformation of the aglycone ring in erythromycin A; however, as such studies are carried out in the solid state, it remains to be shown that this conformation is adopted in solution.

From assigned shift values recorded in both chloroform and pyridine solutions for the various protons and methyl groups of 1, 2, and 3, pyridine Δ-values are calculated and are summarized in Table I. Analysis of the values recorded in this table along with other additional information obtained from their NMR spectra allow the following conclusions to be drawn concerning the configurational and conformational nature of 1, 2, and 3.

TABLE I
Pyridine Δ-Values* (ppm) for Aglycone Ring Protons and Methyl Groups

Compound	H-2	H-3	H-5	H-9	H-11	H-13	2-Me	4-Me	6-Me	12-Me	14-Me
1	-0.30	-0.38	-0.45	-0.66	-0.46	-0.56	-0.25	-0.58	-0.37	-0.16	+0.05
2	-0.27	-0.40	-0.44	-0.38	-0.66	-0.76	-0.25	-0.56	-0.38	-0.17	+0.05
3	-0.29	-0.41	-0.47	-0.40	-0.64	-0.80	-0.23	-0.55	-0.39	-0.29	+0.03

*Δ-Values were calculated from signals measured at 100 MHz at normal probe temperature (ca. 32°C). Sample concentrations were maintained at less than 5% w/v.

Compounds 1, 2, and 3 are Conformationally Homogeneous in Solution, i.e. they adopt the same approximate conformation. --Since it has been previously established that the magnitude of shifts induced in pyridine is dependent upon the relative spatial position of the proton and hydroxyl function under consideration (1,2), the constant Δ-values noted for protons and methyl groups situated at ring positions 2, 3, 4, 5, and 6 (See Table I) conclusively demonstrates that their spatial position relative to nearby polar substituents is the same in 1, 2, and 3. Proton-proton coupling data provide further support for these conclusions since it is observed that the coupling constants for protons at corresponding ring positions (i.e. at 2, 3, 4, 5, 8, 9, 10, 11, 12, and 13) in compounds 1, 2, and 3 are the same (See Table II).

The Configuration of the C-9 Hydroxyl Function is the Same in 2 and 3 and Different from that in 1. --Identical Δ-values (within the limits of measurement error, ± 0.03 ppm) for H-9, H-11, and H-13 in 2 and 3 confirm this point. Since the pyridine shift values for H-11 and H-13 in 2 and 3 are larger than for corresponding protons in 1, and as H-11 and H-13 are situated neither vicinal nor geminal to the C-9 hydroxyl function, 1,3-diaxial and pseudo 1,3-diaxial (9) spatial relationships between the C-9 hydroxyl function and H-11 and H-13, respectively, must be invoked to rationalize these observed shifts (1).

Similar reasoning accounts for the greater deshielding observed for H-9 in 1 than in 2 and 3. The pseudo 1,3-diaxial interaction (9) inferred in 1 presumably exists between H-9 and C-6 OH.

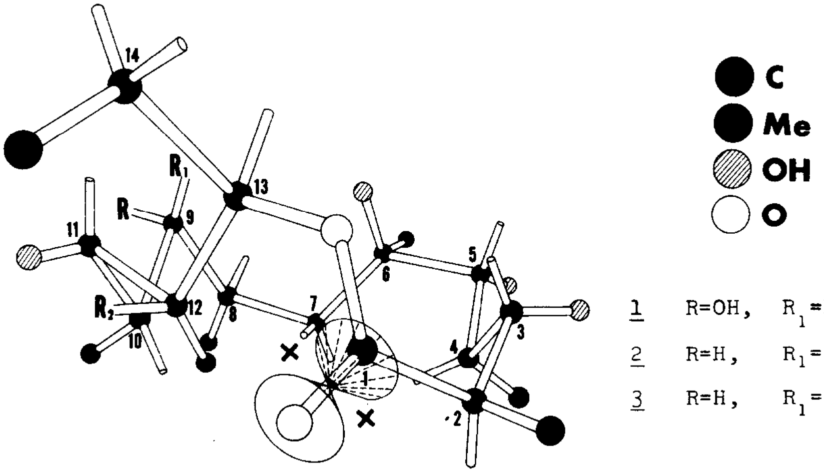
The C-12 Methyl Protons Must Be Located Distant from all Ring Hydroxyl Functions and/or in the Shielding Region of the Lactone Carbonyl Function (10) (See Fig. 1) --This conclusion follows from the observation that the C-12 methyl signals in the spectra of 1, 2, and 3 arise at considerably higher field values than other methyl signals with the exception, in some cases, of the C-14 methyl protons.

Joint consideration of the above solvent shift findings together with dihedral angle estimates (See Table II) for vicinal ring protons, derived from observed proton-proton coupling constants using the Karplus equation (11), shows that the conformation most compatible with all the observed facts is that depicted by the three dimensional ball and stick model in Fig. 1.

TABLE II
Coupling Constants (Hz) and Dihedral Angles for Aglycone Ring Protons

	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{8,9}$	$J_{9,10}$	$J_{10,11}$	$J_{11,12}$	$J_{12,13}$
Obs. Coupling in 1, 2 & 3 (Hz)	10.1	1.0	2.5-1.0	1.8	9.0-10.0	1.0	10.1	4.8
Ø Calc. (deg.)*	0 or 140	70 or 105	60-70 or 105-115	65 or 110	0-15 or 135-140	70 or 105	0 or 140	45 or 125

* For each J value the Karplus equation calculates two possible dihedral angles. Angles underlined are those most compatible with solvent shift findings and are used in deriving aglycone ring conformation. (See Fig. 1)



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2 R=H, R_1=OH, R_2=H
3 R=H, R_1=OH, R_2=OH

Fig. 1. Proposed conformation for 1, 2 and 3 in solution.

This conformation clearly portrays the spatial relationships inferred from solvent studies and coupling data and demonstrates that in 2 and 3, where Δ-values necessitate spatial proximity between the C-9 OH function and H-11 and H-13, the configuration at C-9 must be S (12) (See Fig. 1). In 1 where this is not a necessary requirement the R (12) configuration is assigned to the asymmetry at C-9. It is especially gratifying to note that the configuration in solution as derived from NMR studies is in remarkably good agreement with the conformation previously determined from X-ray studies (8) for the 14-membered lactone ring of erythromycin A.

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