Tetrahedron Letters No.5, pp. 387-390, 1969. Pergamon Press. Printed in Great Britain.

THE CONFORMATION OF ERYTHROMYCIN AGLYCONES

Thomas J. Perun and Richard S. Egan

Research Division, Abbott Laboratories North Chicago, Illinois 60064

(Received in USA 26 September 1968; received in UK for publication 31 December 1968)

Although the structures (1) and stereochemistry (2) of the erythromycin antibiotics (I,II) have been established, and the three-dimensional structure of crystalline erythromycin A hydroiodide determined by X-ray analysis (2a), the conformation in solution of this biologically important molecule was unknown. We can now present experimental evidence, based on nuclear magnetic resonance studies, that the conformation of the macrocyclic ring of the erythromycins in solution is essentially identical with that present in the crystal.

Celmer (3) has proposed an aglycone conformation of the related antibiotic oleandomycin based on the best steric fit of this highly substituted ring to the proposed strain-free conformation of the corresponding 14-membered cyclic hydrocarbon (4). We have found this conformational representation to be consistent with the results of our nmr study of erythromycin aglycones.

A detailed first order analysis of the nmr spectrum of erythronolide B, III (the aglycone of erythromycin B) was possible because of the relatively large difference in chemical shifts of vicinal protons. The use of spin-decoupling techniques permitted the identification of most of the ring proton absorptions and enabled measurement of their respective coupling constants. These assignments are shown in Table I.

The measured values of the observed coupling constants were used to determine approximate dihedral angles between vicinal protons on the erythronolide B ring using the Karplus relationship (5). In the present work correlations were made between coupling constants and dihedral angles using values applicable to six-membered ring systems (6). Thus the large couplings (10-11 Hz) correspond to axial-axial proton relationships in a cyclohexane ring and the small couplings (0-2.5 Hz) correspond to axial-equatorial or equatorial-equatorial relationships. The strain-free conformation of cyclotetradecane contains these same vicinal proton relation-

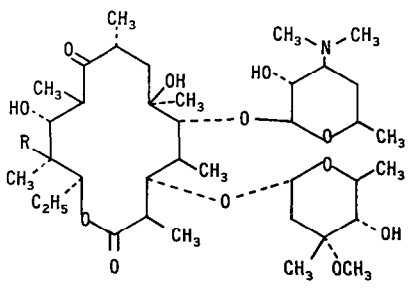
ships, and the proton arrangements in the conformational modification suggested by Celmer (3,4a) provide a reasonable approximation to the experimentally derived model. The close similarity of this conformation (III) to the crystal structure of erythromycin A has been pointed out by Celmer (7) and can best be seen by examination of FMO molecular models (8) of these structures.

TABLE I

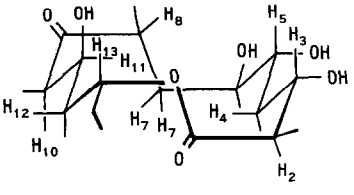
NMR Data for Erythronolide B (III)

Chemical Shifts[a]				Coupling Constants			
H-2	3.00	CH_2-14	1.9-2.3	$J_{2,3}$	10.5	a,a	J_{2},CH_3 6.5
H-3	4.19	CH_3-2	1.52	$J_{3,4}$	<1.0	a,e	J_{4},CH_3 7.0
H-4	2.57	CH_3-4	1.50	$J_{4,5}$	2.5	e,a	J_{8},CH_3 7.0
H-5	4.19	CH_3-6	1.67	$J_{7,7'}$	14.8		J_{10},CH_3 7.0
H-7 }	2.1-2.7	CH_3-8	1.28	$J_{7,8}$	-		J_{12},CH_3 7.0
H-7' }	1.9-2.3	CH_3-10	1.25	$J_{7',8}$	-		J_{14},CH_3 7.0
H-8	3.10	CH_3-12	1.06	$J_{10,11}$	1.0	a,e	
H-10	3.14	CH_3-15	0.90	$J_{11,12}$	10.0	e,e	
H-11	4.42			$J_{12,13}$	<1.0	e,a	
H-12	1.9-2.3			$J_{13,14}$	9.0		
H-13	5.86			$J_{13,14'}$	5.0		

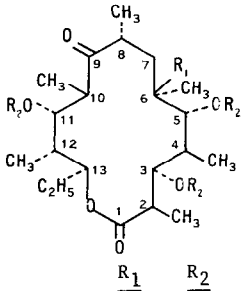
[a]Chemical shifts are reported in ppm (δ) downfield from internal TMS and were determined at 100 MHz in approximately 12% (W/V) solution in pyridine-d_5 at ambient probe temperature (~30°) after the addition of sufficient D_2O (0.02-0.03 ml) to remove -OH resonances. Chemical shifts in this mixed solvent are dependent on the amount of D_2O added.



I R=OH Erythromycin A
II R=H Erythromycin B



Erythronolide B III



	R_1	R_2
III	OH	H
IV	OH	Ac
V	H	H
VI	H	Ac

The observed vicinal coupling constants of the ring protons could represent either a time-averaged spectrum of different conformers in rapid equilibrium, or the spectrum of a single stable conformation. The magnitudes of the coupling constants were indicative of the latter possibility, since in a conformationally mobile ring system, the coupling constants would be time-averaged to intermediate values.

The nmr study was extended to various derivatives of erythronolide B (9) and to erythromycins A and B, as shown in Table II. Comparison of the nmr data of these compounds showed that with the exception of $J_{4,5}$ the coupling constants were invariant throughout the series. This indicated a conformational stability that was not substantially affected by changes in substitution. Table II also shows the invariance of the coupling constants in solvents of differing polarity. No changes were observed in other solvents such as benzene-d_6 or methanol-d_4. The populations of conformers in equilibrium have been shown to be dependent on the dielectric constant of the solvent, as reflected by changes in the time-averaged coupling constants (10). The absence of a solvent effect on coupling constants in the erythronolide series is an indication of the presence of a single conformer.

TABLE II

	Chemical Shifts (δ)					Coupling Constants						
	H-2	H-3	H-5	H-11	H-13	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{10,11}$	$J_{11,12}$	$J_{12,13}$	Other
$CDCl_3$ Solution												
I	~3.0	3.99	3.56	3.83	5.03	10.0	<1	7.0	~1	-	-	
II	~3.0	4.01	3.57	3.81	5.35	9.5	<1	7.2	~1	10.0	~1	
IV	2.79	5.39	4.70	5.13	5.00	11.0	1.3	5.5	1.8	10.0	0.8	
V	2.78	3.88	3.96	3.68	5.16	10.5	<1	~5	2.0	10.0	~1	$J_{5,6}$=2.0
VI	2.81	5.20	4.78	4.89	5.03	10.7	1.5	6.1	1.9	10.0	1.0	$J_{5,6}$=2.0
C_5D_5N Solution												
I	~3.0	4.53	4.02	4.43	5.59	9.0	~1	7.0	1.6	-	-	
II	~3.0	4.40	4.03	4.51	5.78	11.0	~1	7.2	1.5	10.0	~1	
III	3.00	4.19	4.19	4.42	5.86	10.5	<1	2.5	1.0	10.0	<1.0	
IV	2.97	5.72	5.15	5.68	5.25	11.0	1.5	5.2	2.0	9.2	0.8	
V	3.06	~4.2	~4.2	~4.2	5.70	10.0					~1	
VI	2.99	5.57	5.13	5.14	5.23	10.8	1.4	6.4	1.3	10.2	1.5	$J_{5,6}$=2.4

As a further test of conformational homogeneity, the spectra of erythronolide derivatives were examined over a large temperature range (-80° to +110°). The most convenient solvent for high temperature spectra was pyridine-d_5 and no change in coupling constants was observed up to 110°. The chemical shifts were found to be somewhat temperature dependent, probably due to changes in solvent-solute interactions. Methanol-d_4 was the most suitable solvent for low temperature studies. Although increased viscosity of the solutions resulted in significant line-broadening below -50° and caused loss of resolution of the smaller couplings, the larger couplings were unchanged.

The only coupling constant which exhibited any major variation in the compounds reported

here was $J_{4,5}$. Acetylation or glycosidation of the C_3- and C_5- hydroxyls caused an increase in this value over that for the unsubstituted compound. Examination of the FMO models reveals that when these hydroxyls are unsubstituted, their parallel orientation produces an angle between C_4-H and C_5-H which results in a small coupling constant. Substitution of these hydroxyls apparently forces them apart and in turn produces a rotation of the C_4-C_5 bond and an increase in the angle between C_4-H and C_5-H. Two dimensional projections of the X-ray structure of erythromycin A (11) indicate that this relationship between substituents on C_3 and C_5 is also present in the crystal structure.

With the exception of this change in the value of $J_{4,5}$, the measured coupling constants in the spectra of erythromycins A and B are in good agreement with those exhibited by erythronolide B. We feel that this is good evidence that the lactone ring in the parent antibiotic has the same basic conformation as the aglycone.

We have communicated these results to Dr. P. Demarco of the Lilly Research Laboratories who has independently determined the conformation of the lactone ring (accompanying communication). His configurational assignments of the 9-dihydroerythronolides are in agreement with our reported assignments (12).

Acknowledgment

The authors wish to thank Drs. M. I. Levenberg, J. S. Tadanier and P. H. Jones of these laboratories and Dr. P. Beak of the University of Illinois for many helpful discussions.

References

(1) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette and R. Monahan, J. Am. Chem. Soc. 79 6062 (1957)
P. F. Wiley, M. V. Sigal, Jr., O. Weaver, R. Monahan, and K. Gerzon, ibid. 79 6070 (1957)
(2a) D. R. Harris, S. G. McGeachin, and H. H. Mills, Tetrahedron Letters 679 (1965)
(b) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan, and U. C. Quarck, J. Am. Chem. Soc. 78 6396 (1956)
(c) W. D. Celmer, ibid 87 1801 (1965)
(3) W. D. Celmer, Antimicrobial Agents and Chemotherapy 144 (1965)
(4a) W. D. Celmer in Biogenesis of Antibiotic Substances Chapter 10, p. 99, Z. Vanek and Z. Hostalek, ed. Academic Press Inc., New York, N.Y. (1965)
(b) J. Dale, J. Chem. Soc. 93 (1963)
(5) M. Karplus, J. Chem. Phys. 30 11 (1959) M. Karplus, J. Am. Chem. Soc. 85 2871 (1963)
(6) N. S. Bhacca and D. H. Williams, Applications of NMR Spectroscopy in Organic Chemistry p. 49 Holden-Day, Inc., San Francisco, Cal. (1964)
(7) W. D. Celmer in Abstracts of 150th Meeting, American Chemical Society p. 10P (1965)
(8) Framework Molecular Orbital Models, Prentice-Hall, Inc., Englewood Cliffs, N. J.
(9) T. J. Perun, J. Org. Chem. 32 2324 (1967). J. R. Martin and W. Rosenbrook, Biochemistry 6 435 (1967)
(10) R. U. Lemieux and J. W. Lown, Can. J. Chem. 42 893 (1964)
(11) The coordinates were kindly supplied by Professor D. R. Harris
(12) J. R. Martin and T. J. Perun, Biochemistry 7 1728 (1968)