(24) See M. Hall, Jr., "The Theory of Groups", Macmillan, New York, N.Y., 1959, Chapter 19, for a discussion of subgroup lattices.
(25) Note that this will not necessarily be a survey of *all* subgroups of $\mathbf{G}_{192}$ which contain C_2 since the modes in Table I do not correspond to double cosets of C_2 in $\mathbf{G}_{192}$. They are, rather, collections of these double cosets.
(26) "Maximally labeled structure" means a structure in which all four aryl rings are different and none possesses a local C_2 axis.
(27) Related ideas of descriptors and "stereochemical quantum numbers" have been discussed.[28,29]
(28) E. Ruch and I. Ugi, *Top. Stereochem.*, **4**, 99 (1969).
(29) P. Gillespie, P. Hoffman, H. Klusacek, D. Marquarding, S. Pfohl, F. Ramirez, E. A. Tsolis, and I. Ugi, *Angew. Chem., Int. Ed. Engl.*, **10**, 687 (1971).
(30) The number of possible descriptor sets corresponds to the number of isomers, as described ($2 \cdot 3 \cdot 2 \cdot 2^4 = 192$).
(31) Interesting and subtle details of this nomenclature problem depend on the full structure of this subgroup lattice (Figure 2). An unambiguous designation of helicity in isomers differing in S_4 axis orientation is not possible, for the reason that there is no group which acts on the S_4 axis orientation descriptor without acting also on the helicity descriptor. This can be verified by referring to Tables I and V and Figure 2. A group which acts only on the orientation descriptor would have to be of order 6. In the above example, ring 1 was arbitrarily fixed so that the necessary distinction could be made.
(32) (a) Reference 24, Chapters 8 and 9; (b) J. J. Rotman, "The Theory of Groups, An Introduction", Allyn and Bacon, Boston, Mass., 1965, Chapter 6.
(33) It should be pointed out that this will not be an obvious property in the general case. In the tetraaryl systems, the values of the seven descriptors are just the prime factors of the number of isomers ($2 \cdot 3 \cdot 2 \cdot 2^4 = 192$); this occurs because $\mathbf{S}_4[\mathbf{S}_2]$ is a solvable group.[32] In chemical problems involving permutation groups, in particular the symmetric groups $\mathbf{S}_n$ ($n > 5$), this result will not obtain since these groups are not solvable. Group theoretically, the reason for these differences is that there are simple groups which are not of prime order.
(34) P. Finocchiaro, D. Gust, and K. Mislow, *J. Am. Chem. Soc.*, **96**, 3198 (1974).
(35) E. L. Muetterties, *Rec. Chem. Progr.*, **31**, 51 (1970). This article includes an interesting discussion of the group theoretical treatment of a stereochemically disjoint 12-atom polytopal process.
(36) The "effective symmetry group" for these experiments is the group of permutations which do not interconvert any isomers based on the particular substituent pattern. For example, $(Ar_d)_4Z$ structures are invariant to permutations of rings which generate the group $\mathbf{G}_{12a}$.
(37) To be more precise the *upper limit* to the information (mode) obtainable by doing both experiments is determined by the intersection of the effective symmetry groups.
(38) Partial representatives (subgraphs) may however aid in the analysis of such systems; see ref 2.
(39) (a) A. Kerber, "Representations of Permutation Groups. I." Springer-Verlag, New York, N.Y., 1971, p 37; (b) A. Mead, E. Ruch, and A. Schönhofer, *Theor. Chim. Acta*, **29**, 269 (1973).
(40) For example, ref 7. See also D. Gust, P. Finocchiaro, and K. Mislow, *Proc. Natl. Acad. Sci. U.S.*, **70**, 3445 (1973); E. L. Muetterties, *J. Am. Chem. Soc.*, **90**, 5097 (1968); **91**, 1636 (1969); M. Gielen and C. Depasse-Delit, *Theor. Chim. Acta*, **14**, 212 (1969); M. Gielen and J. Topart, *ibid.*, **18**, 7 (1969).
(41) "Topological representations" in the chemical literature are actually representations of double coset matrices or sums of double coset matrices for permutational problems of this kind; that is, a double coset matrix is an incidence matrix for the topological representation.[19]
(42) H. Wielandt, "Finite Permutation Groups", Academic Press, New York, N.Y., 1964, Chapter 1.
(43) W. G. Klemperer, *J. Am. Chem. Soc.*, **94**, 6940, 8360 (1972).
(44) Here the nonself-inverse modes are considered together so that the connectivity for $[3a,3b]$ is $16/2 = 8$. For mode $3a$ alone the topological representation would be directed.

Conformational Flexibility of Erythronolide B, the 14-Membered Aglycon Ring of the Erythromycins[1]

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Abstract: Detailed analyses of ^{1}H NMR and CD spectra of derivatives of erythronolide B have revealed that this aglycon ring is conformationally flexible. The general diamond-lattice type conformation is maintained with subtle modification in either the C-2 through C-5 or C-6 through C-9 ring segments depending on the nature and position of ring substituents. Variable temperature measurements in a series of *O*-acetyl derivatives have indicated that the conformational changes in these two regions are interdependent and enabled the determination of the limiting conformations which are populated to different extents in this series.

The erythromycin antibiotics are representative members of a group of natural products which possess unique structural and conformational characteristics. The aglycon of these glycosidic compounds, a 14-membered macrocyclic ring, possesses surprising conformational stability which is evidenced by its spectroscopic properties and the nature and selectivity of its chemical reactivity. Previously reported studies in these laboratories[3] using ^{1}H NMR and CD data have shown the conformation of erythronolide B (**1**) to be that depicted in Figure 1. These studies have indicated that the stability of this conformation is probably a consequence of the adoption of a diamond-lattice type framework of the ring atoms. In addition, the unique substitution pattern of this macrocycle confers additional rigidity by minimizing internal interactions and at the same time preventing free rotation of carbon–carbon bonds.

Throughout the series of derivatives studied, it was clear that a large degree of conformational homogeneity existed as evidenced by the consistency of proton chemical shifts and coupling constants. As more and more members of the series were examined, however, it became apparent that subtle conformational effects were occurring as a result of certain substituent changes on the macrocyclic ring. It was felt that a more detailed scrutiny of the ^{1}H NMR and CD spectra of these compounds and the use of the more recent ^{13}C NMR spectroscopic technique would provide useful information about these subtle changes. This paper discusses the studies carried out using ^{1}H NMR and CD instrumentation.

Experimental Section

The ^{1}H NMR spectra which will be discussed in the following sections were obtained on a Varian Associates HA-100 spectrometer operating in frequency sweep. Chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane, and coupling constants are reported in hertz. Both chemical shifts

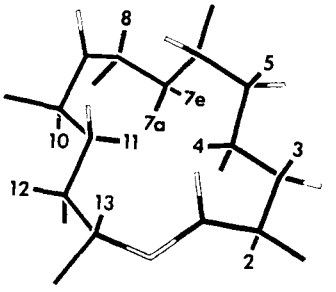


Figure 1. Photograph of a molecular model of the conformation of erythronolide B showing the syn-periplanar relationship between the 3- and 5-hydroxyls. Solid lines represent C-C and C-H bonds, while outlined lines represent C-O bonds. The position of ring protons is given by the appropriate numbers. Protons associated with methyl and hydroxyl groups are not shown for clarity.

Table I. Variation of $J_{4,5}$

	$J_{4,5}$	$J_{5,6}$		$J_{4,5}$	$J_{5,6}$
Erythronolide B					
Unsubstituted (1)	2.9		3,5-Diacetyl (6)	4.6	
3-Acetyl (3)	3.1		3,5,11-Triacetyl (7)	5.6	
5-Acetyl (4)	3.5				
11-Acetyl (5)	2.0				
6-Deoxyerythronolide B					
Unsubstituted (2)	2.5	4.7	3,5-Diacetyl (11)	7.4	1.8
3-Acetyl (8)	2.8	5.2	3,5,11-Triacetyl (12)	6.2	2.0
5-Acetyl (9)	4.2	2.3			
11-Acetyl (10)	2.2	4.5			

and coupling constants were measured directly from the spectra using first-order rules.

Circular dichroism spectra were obtained at 29° (cell compartment temperature), unless noted otherwise, in a Durrum-Jasco ORD/UV/CD 5 instrument operating under constant nitrogen flush.

Discussion

1. Variation in $J_{4,5}$. The conformation of erythronolide B (**1**) (Figure 1) places the 3- and 5-hydroxyl groups in a syn-periplanar relationship which then requires that the orientations of H-4 and H-5 be equatorial and axial, respectively. The magnitude of $J_{4,5}$ in erythronolide B or 6-deoxyerythronolide B is small (2.9 and 2.5 Hz, respectively), consistent with this assignment (Table I).

When either the 3- or 5-hydroxyl group is acetylated, the magnitude of $J_{4,5}$ in the resulting monoacetates **3, 4, 8,** and **9** is unchanged. However, when both the 3- and 5-hydroxyl groups are acetylated in either the diacetates **6** or **11,** or in the triacetates **7** or **12,** the magnitude of $J_{4,5}$ is markedly increased to between 5 and 6 Hz. There is a concurrent decrease in the magnitude of $J_{5,6}$ to approximately 2 Hz in the 6-deoxy compounds **7** and **12** (Table I).

The conformational alteration associated with the changes in the magnitude of the coupling constants is not unexpected as, in the diacetates or triacetates, no stabilizing hydrogen bond is possible between the 3- and 5-substituents.[3] In addition, the bulk of the syn-periplanar substituents will destabilize the conformation and require some reorganization to minimize this interaction.

Since solution of the Karplus equation yields two possible dihedral angles, the changes in coupling constants can be accommodated by two opposite sets of bond rotations. The NMR data do not allow differentiation between these sets of rotations; however, by analogy to X-ray data of the intact antibiotic,[4] one reorganization is favored.[5a,b]

Upon diacetylation, the 3- and 5-oxygen atoms move apart by a rotation of the 4–5 bond in a manner that moves

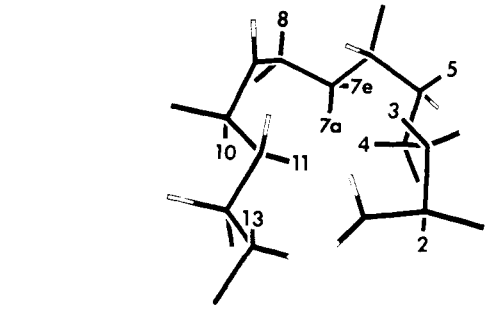
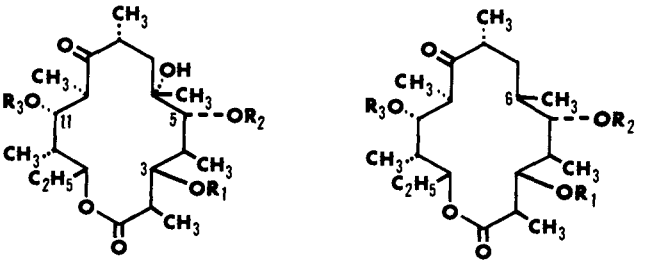


Figure 2. Photograph of a molecular model of the conformation of 3,5-disubstituted erythronolide B showing the conformational reorganization of the C2-C6 region.



1 $R_1 = R_2 = R_3 = H$

2 $R_1 = R_2 = R_3 = H$

3 $R_2 = R_3 = H$, $R_1 = Ac$

8 $R_2 = R_3 = H$, $R_1 = Ac$

4 $R_1 = R_3 = H$, $R_2 = Ac$

9 $R_1 = R_3 = H$, $R_2 = Ac$

5 $R_1 = R_2 = H$, $R_3 = Ac$

10 $R_1 = R_2 = H$, $R_3 = Ac$

6 $R_3 = H$, $R_1 = R_2 = Ac$

11 $R_3 = H$, $R_1 = R_2 = Ac$

7 $R_1 = R_2 = R_3 = Ac$

12 $R_1 = R_2 = R_3 = Ac$

the 5-oxygen "down" (Figure 2). This effectively increases the dihedral angle between H-4 and H-5 and, as the rotation is expected to increase the angle beyond 90°, thereby increases the magnitude of $J_{4,5}$. The decrease in the magnitude of $J_{5,6}$ requires that the 5–6 bond also be rotated but not to the same extent as the 4–5 bond since the resulting dihedral angle is near the 90° minimum in the Karplus curve as revealed by the small $J_{5,6}$ coupling. These rotations relieve the syn-periplanar interaction between the 3- and 5-acetoxyl groups and decrease the gauche interaction between the 6-methyl and 5-acetoxyl groups.

It should be stressed that the reorganization appears to be limited to the portion of the aglycon ring in which the 5-substituent is situated since no other coupling constants are affected.

The opposite rotation (i.e., the 3-oxygen moves "down" while the 5-oxygen moves "up") could have the same effect on the coupling constants. In fact, these rotations might seem to be preferred in that examination of molecular models reveals that the required rotation of the 4–5 bond is such that the oxygen atoms are directed away from the methyl groups on the adjacent carbons rather than approaching an eclipsed conformation. However, while a more favorable situation exists between vicinal substituents under these circumstances, the 4-methyl group is rotated toward an internal position and a 6-methyl-8-methyl syn-periplanar interaction is formed. The 4-methyl group is rotated away from the center of the ring and the syn-periplanar interactions are further decreased in the favored reorganization. A similar reorganization is evident when other substituents are present in the 3- and 5-positions, e.g., benzoyl groups and 6-deoxy sugars.[5b]

Table II. Circular Dichroism Spectra (CH_3OH Solvent)

Derivative	Ketone	Lactone
Erythronolide B		
Unsubstituted (1)	−12180	−4260
3-Acetyl (3)	−11625	±0
5-Acetyl (4)	−10750	−1235
3,5-Diacetyl (6)	−11100	+3170
3,5,11-Triacetyl (7)	−7260	+4250
11-Acetyl (5)	−17825	−3200
6-Deoxyerythronolide B		
Unsubstituted (2)	−17480	−5420
3-Acetyl (8)	−17130	−2320
5-Acetyl (9)	−16370	−5300
11-Acetyl (10)	−17400	−3815
3,5-Diacetyl (11)	−16260	−1306
3,5,11-Triacetyl (12)	−8920	+4615

In principle, a conformational alteration involving movement of C-3 and/or C-5 could be transmitted to either the ketone or the lactone chromophore and be detected in the CD spectra. Because C-3 is rather close to the lactone chromophore, and, indeed, is the sign-determining atom in the Beecham–Wolf treatments,[6,7] such changes are most likely to appear in the lactone signals. Reference to Table II shows that this is the case.

With the exception of substances acetylated at both C-3 and C-5, the lactone bands are uniformly negative. The overall amplitudes are not large compared with those seen in other studies of lactones,[8] suggesting that the chromophore is not very twisted and is in an environment which is nearly symmetrically surrounded by functional groups.

Monoacetylation of either C-3 or C-5 results in a diminution in lactone intensity, with the C-3 effect being the larger. This is easily accommodated as this group is not only the closest to the chromophore but is actually the sign-determining atom in the Beecham–Wolf treatments[6,7] so that substituent changes here could not fail to be significant determinants of lactone amplitudes.

Both C-3 and C-5 lie rather close to the lactone plane and, in the Klyne[8] or Snatzke[9] treatments, the increased steric bulk attending acetylation could bring the affected function forward so as to make either a nodal or an antioctant contribution, thus explaining the data. Whether one prefers the Beecham–Wolf or the Snatzke–Klyne treatments, one can accommodate the CD data for **1** and **2** as well as the monoacetates **3, 4, 8,** and **9** without invoking any conformational movement of any significance.

In contrast to the monoacetyl derivatives, the CD spectra of the 3,5-diacetyl analogs (Table II, **6** and **11**) show either strongly decreased amplitude (**11**) or sign inversion (**6**). The corresponding ketone peaks, on the other hand, are relatively unaffected. The 6-deoxy spectra, in particular, give strong evidence for a conformational change. The observed amplitude change (+4114) is considerably larger than that calculated from the incremental contributions of C-3 and C-5 acetylation alone (+3220 = 3100 + 120). In the erythronolide B series, the evidence is not, however, as convincing because the calculated value (+7285 = 4260 + 3025) is only slightly different than the experimental value (+7430). With the triacetyl analogs, the 6-deoxy series provides, again, the most convincing evidence of conformational change. For example, 3,5,11-triacetyl-6-deoxyerythronolide B undergoes a change in lactone amplitude of +10035 (relative to 6-deoxyerythronolide B) compared with a calculated value of +4825 (3100 + 120 + 1605). On the other hand, 3,5,11-triacetylerythronolide B shows a change of +8510 which compares with a calculated value of +8345 (4260 + 3025 + 1060). Thus, under the conditions of the CD measurements, there is clear evidence for conformational shift in the 6-deoxy series. It is interesting to note that the effect on the coupling constants is also greater in the 6-deoxy series.

Table III. Anomalous NMR Parameters

Erythronolide B	3-Acetyl (3), 5-acetyl (4), 3,5-diacetyl (6)	Unsubstituted (1), 3,5,11-triacetyl (7)	11-Acetyl (5)
Chemical shift H-8	2.74, 2.72, 2.74	—, 2.88	3.30
Coupling constants			
$J_{7a,8}$	9.2, 9.8, 9.4	6.7, 6.4	3.4
$J_{7e,8}$	4.0, 3.2, 3.3	7.4, 5.5	11.5

2. Variation of the Chemical Shift of H-8 with $J_{7a,8}$ and $J_{7e,8}$. Conformational Equilibrium. The NMR spectra of the 11-acetyl esters of erythronolide B and 6-deoxyerythronolide B, **5** and **10,** are anomalous in that they show the resonance of H-8 at 3.30 and 3.14 ppm in $CDCl_3$ solution. These resonances are further downfield than the resonances of the corresponding proton in any other acetate derivative by approximately 0.6 and 0.3 ppm, respectively (Table III). Since H-8 is distant from the 11-acetoxyl group in the Perun conformation (Figure 1), and the shift is significantly large, an explanation for this phenomenon was sought. Because the spectra of the 6-deoxy derivatives are less well characterized because of more extensive chemical-shift overlap and additional couplings arising from H-6, the following discussion is predominantly concerned with derivatives of erythronolide B.

The effect responsible for the anomalous chemical shift of H-8 cannot be attributed solely to the absence of an 11-hydroxyl hydrogen bond to the ketone and/or lactone carbonyl since the chemical shifts of H-8 in the spectra of the triacetyl derivatives **7** and **12,** also devoid of an 11-hydroxyl group, are only slightly further downfield with reference to the chemical shifts in the nonacetylated derivatives **1** and **2.** Since the anomalous chemical shift is observed in both the normal and 6-deoxy derivatives, explanations requiring the participation of the 6-hydroxyl group such as hydrogen bonding, enolization, or hemiacetal formation are untenable. The possibility of epimerization at C-8 under acid conditions[10] via an elimination–readdition sequence was removed when 11-acetylerythronolide B was converted to normal 3,5,11-triacetylerythronolide B by direct room-temperature acetylation with acetic anhydride in pyridine.[11] these considerations pointed to the possibility that a conformational change had occurred in the C-9,C-8 region of the aglycon ring which was responsible for the chemical-shift change since other explanations could not satisfy all the data.

A second observation was made which confirmed that a conformational change had occurred in the acetate esters. While most coupling constants remain relatively constant throughout the series, with the exception of $J_{4,5}$ discussed elsewhere, the magnitudes of $J_{7a,8}$ and $J_{7e,8}$ vary considerably. Based on these coupling constants, the compounds can be segregated into three different categories in $CDCl_3$ solution (cf. Table III).

(1) Extreme coupling constants are observed in the spectra of 3-acetyl- (**3**), 5-acetyl- (**4**), and 3,5-diacetylerythronolide B (**6**).

(2) Intermediate coupling constants are observed in the spectra of erythronolide B (**1**) and 3,5,11-triacetylerythronolide B (**7**).

(3) Opposite extreme coupling constants are observed in the spectra of 11-acetylerythronolide (**5**).

Since the coupling constants in the last category are similar in magnitude to those of the first category but assigned to opposite 7-methylene protons, it could be theorized that

Table IV. Temperature Dependence of Coupling Constants of Erythronolide B and Selected Esters[a]

	Erythronolide B (1)		3,5,11-Triacetyl-erythronolide B (7)	
	Ambient	110°	Ambient	110°
$J_{2,3}$	10.4	10.2	11.4	11.0
$J_{7a,7e}$	14.2	14.5	15.5	15.6
$J_{7a,8}$	7.4	6.7	8.3	6.4
$J_{7e,8}$	6.4	7.4	3.2	5.5
$J_{11,12}$	10.0	9.8	9.6	9.5
	11-Acetyl-erythronolide B (5)		3,5-Diacetyl-erythronolide B (6)	
	Ambient	110°	Ambient	110°
$J_{2,3}$	10.0	10.1	11.1	10.9
$J_{7a,7e}$	14.4	14.5	14.7	14.6
$J_{7a,8}$	2.6	3.4	9.6	9.4
$J_{7e,8}$	12.4	11.5	3.3	3.3
$J_{11,12}$	9.9	9.6	10.1	10.3

[a] Measured from C_5D_5N spectra at 100 Hz sweep width. Because of natural line-width data accurate to ±0.2 Hz.

the 7-methylene protons have reversed chemical shifts with no change in coupling constants. This possibility is not probable, because the very small differences in the chemical shifts would require fortuitously large but opposite changes.

In all cases, the chemical shift of H-8 in $CDCl_3$ solution can be correlated with the magnitude of the 7-methylene proton coupling constants (Table III). The extreme categories of coupling constants correlate with extreme values of chemical shifts, while the compound having intermediate coupling constants similarly has an intermediate chemical shift.

The three categories of data can reflect either the presence of three distinct, stable conformations or could be indicative of a shift in the relative populations of two (or more) limiting conformations involved in a facile conformational equilibrium. That these three categories do not represent three distinct, stable conformations but rather are indicative of a shift in a conformational equilibrium was demonstrated by the temperature dependence of the 7-methylene proton coupling constants.

The temperature dependence of $J_{7a,8}$ and $J_{7e,8}$ combined with the invariance of other coupling constants under the same conditions (Table IV) revealed that the conformational equilibrium was restricted to the 7-methylene region of the macrolide ring. The relative populations of the two limiting conformations vary between the three categories. One limiting conformation is heavily populated in the case of **3, 4,** and **6,** while the population of the second limiting conformation is small. In the case of **1** and **7,** both conformations are nearly equally populated and, in the case of **5,** only the second limiting conformation is significantly populated. The observed chemical shifts and coupling constants are time averaged by a rapid equilibrium between the limiting conformations which can be likened to a pseudorotation restricted to only a portion of the macrocyclic ring.

The conformational equilibrium is such that the magnitude of $J_{7a,8}$ decreases while $J_{7e,8}$ increases in going from one limiting conformation to another. If a (Figure 3) represents the totally staggered Perun conformation in which large $J_{7a,8}$ coupling constants are predicted (limiting conformation) as proposed for **3, 4,** and **6** as well as **2** and **11,** then two diametrically opposite conformational reorganizations are possible in which the coupling constants will reverse in magnitude as proposed for **5.** Intermediate values in **1** and **7** can be accommodated by a time-averaged combination of conformation a and one other.

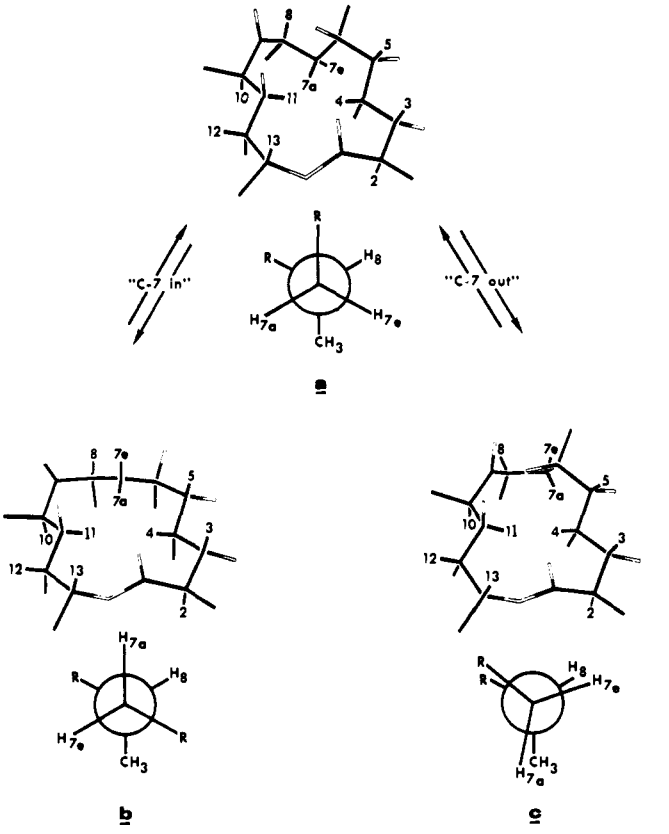


Figure 3. Conformational equilibrium of erythronolide B and derivatives. Photographs of molecular model constructions and partial Newman projections (R = remainder of ring) of three possible limiting conformers: (a) totally staggered conformation; (b) "alternate diamond lattice" conformation; and (c) partially eclipsed conformation.

In a (Figure 3), the large $J_{7a,8}$ and small $J_{7e,8}$ coupling constants arise from interactions between protons at dihedral angles of approximately 180 and 60°. The dihedral angle between H-7a and H-8 can be decreased by an "inward" displacement of C-7 which results in an increase in the corresponding dihedral angle between H-7e and H-8 until, at the limit, the dihedral angles are 60 and 180° (b, Figure 3). This conformation is the "alternate diamond lattice" conformation[3] which without further reorganization, precluded by the invariance of other coupling constants, is thermodynamically unfavorable because of syn-periplanar methyl interactions.

A second possible rearrangement via an "outward" displacement of C-7 decreases the angle between H-7a and H-8, while the corresponding angle between H-7e and H-8 also decreases until, at the limit, the dihedral angles are 120 and 0° (c, Figure 3). This conformational reorganization creates no additional unfavorable interactions save the eclipsing of the 7-methylene protons with the substituents on C-8 and C-6 in a manner analogous to the formation of a five-membered ring. This rearrangement also does not require that bulky groups eclipse one another during the reorganization, and therefore no high-energy barrier is expected to exist between conformations a and c.

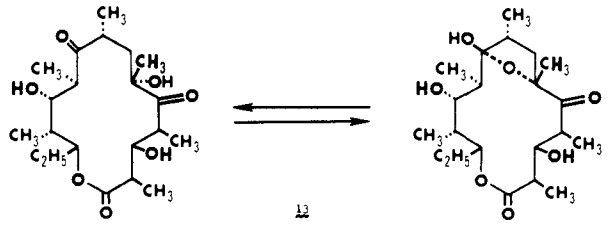
In c, H-8 is no longer eclipsed by the α-carbonyl group but has moved slightly "downward". The 0.63-ppm downfield shift associated with the conformational change from a to c is not unreasonable as Karabatsos[12] working with aliphatic aldehydes has attributed a 0.69-ppm shift of an α-proton resonance to a similar conformational change.

A related effect has been noted in 5-deoxy-5-oxoerythronolide B **13** which, in $CDCl_3$ solution, has been shown by

Table V. Effect of Temperature on Ketone Cotton Effect

Substrate	Solvent	T, °C	$[\theta]$	Variation, %
Erythronolide B (1)	Isopropyl alcohol	30	−13895	
Erythronolide B (1)	Isopropyl alcohol	55	−13987	+0.7
Erythronolide B (1)	Isopropyl alcohol	80	−14801	+6
Erythronolide B (1)	Dioxane	30	−14973	
Erythronolide B (1)	Dioxane	55	−14760	−1.4
Erythronolide B (1)	Dioxane	80	−14542	−3
3-Acetylerythronolide B (3)	Isopropyl alcohol	30	−10658	
3-Acetylerythronolide B (3)	Isopropyl alcohol	55	−10696	+0.4
3-Acetylerythronolide B (3)	Isopropyl alcohol	80	−11258	+5
3-Acetylerythronolide B (3)	Dioxane	30	−12681	
3-Acetylerythronolide B (3)	Dioxane	55	−12498	−1.4
3-Acetylerythronolide B (3)	Dioxane	80	−12519	−1.3
11-Acetylerythronolide B (5)	Isopropyl alcohol	30	−18146	
11-Acetylerythronolide B (5)	Isopropyl alcohol	55	−17795	−2
11-Acetylerythronolide B (5)	Isopropyl alcohol	80	−17796	−2
11-Acetylerythronolide B (5)	Dioxane	30	−17040	
11-Acetylerythronolide B (5)	Dioxane	55	−16210	−5
11-Acetylerythronolide B (5)	Dioxane	80	−15660	−8
1-(−)-Camphor	Isopropyl alcohol	30	−5184	
1-(−)-Camphor	Isopropyl alcohol	55	−5403	+4
1-(−)-Camphor	Isopropyl alcohol	80	−5656	+9
1-(−)-Camphor	Dioxane	30	−4860	
1-(−)-Camphor	Dioxane	55	−4901	−1
1-(−)-Camphor	Dioxane	80	−5011	−3
1-(−)-Fenchone	Isopropyl alcohol	30	−2963	
1-(−)-Fenchone	Isopropyl alcohol	55	−2954	−0.3
1-(−)-Fenchone	Isopropyl alcohol	80	−2994	+1
1-(−)-Fenchone	Dioxane	30	−2669	
1-(−)-Fenchone	Dioxane	55	−2612	−2
1-(−)-Fenchone	Dioxane	80	−2643	−1

NMR spectroscopy[13] to exist in a hydroxy ketone–hemiacetal equilibrium as previously suggested.[14] This equilibrium



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is quite analogous to that proposed for the erythronolide B acetate esters in that a close approach of the 6-hydroxyl group to the 9-ketone for hemiacetal formation requires a conformation reorganization identical with the a to c (Figure 3) equilibrium.

In addition, a number of acid-catalyzed rearrangements of erythronolide B[10] and the erythromycins[15-19] are known to proceed via a hemiacetal or hemiacetal–enol ether pathway.

The driving force for such a conformational change in the acetates remains unclear as does the effect responsible for a shift in the conformational equilibrium. It is interesting to note, however, that only the region of the aglycon ring displaced from the "alternate diamond lattice" conformation is involved. The remainder of the ring remains rigid. This is clearly shown in part by the similarity of the remaining coupling constants and evidence that conformation c is assumed by both 11-acetylphenylboronates (cf. Table 8 of previous communication[3]) in which the orientation of the 3- and 5-hydroxyl groups are fixed.

It appears the two conformational effects discussed, that involving $J_{4,5}$ and that involving the 7-methylene proton couplings with H-8, are interrelated. When the 11-hydroxyl group is acetylated either bond-angle changes, dipolar interactions, or perturbation of hydrogen-bonding characteristics, or a combination of effects introduce a conformational instability. This instability is reflected by a shift in the conformational equilibrium toward c (Figure 3) when the 3- and 5-hydroxyls are unsubstituted and hydrogen bonding maintains their relative syn-periplanar orientation. However, when these hydroxyl groups are also substituted by acetyl groups, the conformational change leading to an increase in the magnitude of $J_{4,5}$ appears to be sufficient to relieve the instability, and no shift in the conformational equilibrium is apparent.

The effect on the ketone CD spectra of various erythronolide derivatives of acetylation at C-11 is quite varied (Table II). With 11-acetylerythronolide B, a sizeable increase in negative amplitude is seen. No amplitude change is seen with 11-acetyl-6-deoxyerythronolide B. Thus substitution of a hydroxyl function by a larger acetoxy function at C-11 has a variable effect on ketone amplitude. No similar diversity of outcome is seen on acetylation of any other hydroxyl in these macrolides. In the Perun conformation, the 11-hydroxyl group is axial. Acetylation might be expected to introduce some conformational instability into such a highly substituted molecule and result in conformational reorganization. It was first thought that this must involve a forward movement of C-11, because the hydroxyl (acetoxyl) function at that center is in a negative octant and can be brought successively into a nodal zone and then into a forward, oppositely signed octant by outward rotation. Detailed NMR examination showed, however, that this explanation was not likely. The only coupling constants which change significantly were those near C-7. This meant that increased bulk at C-11 triggers a conformational reorganization across the ring. It is now clear that this effect is responsible for the CD amplitude effects, and that the change results in increased twisting in some cases and decreased twisting in others, and this is what the CD measurements are reflecting.

In light of these clear indications of conformational differences, it was of special interest to see if variable-temperature CD measurements would detect conformational equilibration analogous to that seen in the variable-temperature NMR work.

Table V lists data obtained for erythronolide B in isopro-

pyl alcohol and dioxane over the temperature range of 30–80°. Camphor and fenchone were included as rigid model substances in order to control the magnitude of amplitude changes to be expected from nonconformational causes. In isopropyl alcohol, an amplitude change of up to 10% can be experienced without indicating that a conformational alteration is taking place. In dioxane, a solvent where asymmetric solvation is much less likely to occur, a nonspecific solvational effect of 3% can be encountered. Under the conditions of these measurements, the amplitude changes observed with erythronolide B (**1**) are too small to be due to conformational movement. NMR studies in pyridine-d_5 solutions suggested that some conformational change can take place with this substance at elevated temperatures. This difference in outcome is likely attributable to the different conditions used. NMR studies indicated that 3-acetylerythronolide B (**3**) does not undergo a conformational alteration. The data in Table V clearly show that this molecule also fails to undergo conformational flexure detectable by CD measurements at various temperatures. On the other hand, NMR measurements clearly show a fairly substantial conformational change at elevated temperatures for 11-acetylerythronolide B (**5**), and the data in Table IV show that this effect is also detectable in dioxane through CD measurements. It is interesting to note, however, that no such movement is detectable in isopropyl alcohol. Variable-temperature CD work, then, confirms the NMR-based proposal that acetylation at C-11 induced a fairly deep-seated conformational instability in some derivatives and helps to define some of the conditions under which this change takes place.

Summary

In summary, this study has shown that there are two regions of the erythronolide macrocyclic ring that are prone to conformational changes induced by ring substituents. One is the region where the glycosidic moieties are attached in the parent erythromycin compounds (C-2 thru C-5 ring segment), and the changes are a consequence of the presence or absence of through-space interactions between such substituents. The other conformational effects discussed with reference to certain ester derivatives are manifested in a region which has undergone displacement from the favored diamond lattice conformation (C-6 thru C-9 ring segment). The relative freedom of this region results in the establishment of an equilibrium between two limiting conformations which vary in population among members of the series of derivatives. The subtle nature of these conformational changes made it desirable to study them with ^{13}C NMR techniques as part of the on-going application of ^{13}C NMR spectroscopy to macrolide compounds.[20]

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