Tetrahedron. Vol. 29, pp. 2525 to 2538. Pergamon Press 1973. Printed in Great Britain

THE CONFORMATION OF ERYTHRONOLIDE, THE 14-MEMBERED AGLYCONE RING OF THE ERYTHROMYCIN ANTIBIOTICS[1]

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(*Received in the USA* 11 *December* 1972; *Received in the UK for publication* 2 *April* 1973)

Abstract—The solution conformation of erythronolide B and derivatives has been deduced from NMR and CD spectral data. The conformation is basically a "diamond lattice" type in which the ring atoms occupy cyclohexane-like positions but slightly modified to incorporate the lactone and ketone groups and to relieve an unfavorable *syn*-periplanar interaction. Variable temperature experiments revealed that erythronolide B and derivatives do not undergo facile ring inversion or pseudorotation and suggested the presence of a single stable conformation. Several features of the proposed conformation were demonstrated by the NMR and CD spectra of suitable derivatives. These features included the *syn*-periplanar relationship between the 3- and 5-OH groups, the axial orientation of the 11-OH group, and the proximity of the 6-OH group to the 9-ketone.

The conformational analysis of medium and large ring systems is an area of increasing investigation and interest.[6,7] 14-Membered rings have been studied in cases of saturated hydrocarbons[8,9] and cyclic peptides[10,11] as well as in other classes of compounds.[12] These studies have generally built on the foundation laid by Dale both in theoretical[13,14] and experimental[8,9] studies of representative 14-membered ring compounds.

An earlier conformational model for macrolide antibiotics was proposed by Celmer[15] which was based on Dale's "diamond lattice" conformation for cyclotetradecane. The considerations leading to this proposed conformation have been discussed in previous communications. Our initial goal was to determine experimentally by nuclear magnetic resonance and circular dichroism techniques if there was a single conformation present in solution, and if the data agreed with the proposed model. During the course of this work, a modified conformation for the erythromycin aglycone ring evolved,[2-5] and a new "diamond lattice" conformational model was proposed.[4,16]

Due to the complexity of the NMR spectra of the intact erythromycin antibiotics, initial work centered on several simple aglycone compounds which are biogenetic precursors and shunt metabolites isolated previously by Martin and others.[17-20] Specifically, an analysis is presented of the 100 and 220 MHz NMR spectra of erythronolide B (1),[2] 6-deoxyerythronolide B (2),[2,4] and their acetate esters 3–12 (Ac = CH_3CO used throughout)[2,21] as well as several other derivatives.[2,3,4]

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EXPERIMENTAL

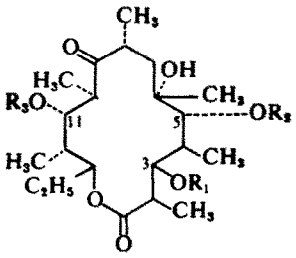
The 100 MHz NMR spectra were obtained on a Varian Associates HA-100 spectrometer operating in frequency sweep. The 220 MHz spectra were obtained on a Varian Associates HR-220 spectrometer at the Applications Laboratory, Varian Associates, Palo Alto, California, by Mr. Lewis Cary. Spin decoupling experiments at 100 MHz were performed with an impedance matched Hewlett-Packard audio-oscillator, Model 200 AB. Chemical shifts are reported in ppm (δ) downfield from internal tetramethylsilane and coupling constants are reported in Hz. Both chemical shifts and coupling constants were measured directly from the spectra using simple first order rules. Higher order effects were disregarded since the dispersion of the chemical shifts of interacting protons was generally large, especially at very high field.

CD spectra were obtained using a Durrum-Jasco ORD/UV/CD 5 instrument operating at 29° (cell compartment temperature) unless specified otherwise. The optical train was kept under constant flush with dry N_2 gas. MeOH (Baker, Spectroscopic Grade) was used as the solvent except where otherwise noted.

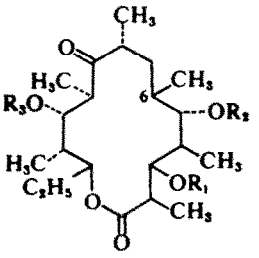
RESULTS AND DISCUSSION

A. *Conformational homogeneity*

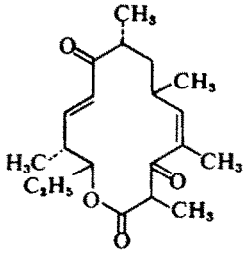
The conformational assignment made from the NMR spectra of these compounds depends to a large extent on the values of the vicinal coupling constants of ring protons. However, the observed coupling constants as well as the chemical shift data could represent either a time averaged spectrum of the different conformers in rapid equilibrium (pseudorotation), or the spectrum of a single stable conformation.[22] Before detailing the conformation proposed for these compounds, the results of several experiments which indicated that a single



1: $R_1 = R_2 = R_3 = H$
3: $R_2 = R_3 = H, R_1 = Ac$
4: $R_1 = R_3 = H, R_2 = Ac$
5: $R_1 = R_2 = H, R_3 = Ac$
6: $R_3 = H, R_1 = R_2 = Ac$
7: $R_1 = R_2 = R_3 = Ac$
14: $R_1 = R_2 = R_3 = Bz$



2: $R_1 = R_2 = R_3 = H$
8: $R_2 = R_3 = H, R_1 = Ac$
9: $R_1 = R_3 = H, R_2 = Ac$
10: $R_1 = R_2 = H, R_3 = Ac$
11: $R_3 = H, R_1 = R_2 = Ac$
12: $R_1 = R_2 = R_3 = Ac$



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stable conformation was present should be given. Because of the relative insolubility of erythronolide B in most non-polar solvents, the most extensive study was carried out with 3,5,11-triacetylerythronolide B (7).

As a test of conformational homogeneity, the spectra of 7 were examined between −80 to +110°. The methine protons on C-3, C-5, C-11, and C-13 were of major significance in this study since they had distinctive splitting patterns, were well separated from other resonances due to oxygenated substituents and showed minimal chemical shift overlap. The most convenient solvent for the high temperature spectra was pyridine-d_5 and no significant change in the coupling constants of H-3, H-5, H-11, or H-13 was observed up to 110°. The apparent resolution improved at higher temperatures because of decreased solvent viscosity and increased random tumbling.[23] The chemical shifts were found to be temperature dependent, probably due to changes in solvent-solute interactions. For example, the chemical shift of H-13 changed from 5·25 ppm at ambient temperature to 5·20 ppm at 110°. Methanol-d_4 was the most suitable solvent for low temperature studies and no significant change in coupling constants of H-3, H-5, H-11, or H-13 was observed down to −50°. Below this temperature, the increased viscosity of the solutions resulted in significant line broadening which caused a loss in resolution of the smaller couplings. However, determination of the large couplings such as $J_{2,3}$ was still possible even at −80°, and no significant change in their magnitudes was evident.

Molecular flexibility can also be detected by use of variable temperature circular dichroism measurements.[24] In moderately flexible molecules, amplitude differences are noted for the Cotton effect originating in light induced electronic transitions of the ketonic CO moiety. In intermediate cases, sigmoidal Cotton effects are observed in which contributions from both limiting conformations can be recognized. In extreme cases, actual sign inversions have been recorded. Accordingly, similar studies were carried out on certain erythromycin derivatives. Camphor and fenchone were included in the study as examples of rigid molecules for which conformational flexibility cannot be invoked. These substances are suitable for controlling amplitude differences which are caused by other phenomena than conformational movement. Over the temperature range of 30 to 80°, it was found that the amplitude changes observed for erythronolide B (1) were small when dioxane and isopropanol are used as solvents. Under no conditions were effects seen of greater magnitude than those observed for the control terpenes.

The invariance of the coupling constants of 7 in solvents of widely differing polarities is demonstrated in Table 1. The populations of conformers in equilibrium have been shown to be dependent on

Table 1. Chemical shifts and coupling constants of 3,5,11-triacetylerythronolide B (1) in various solvents[a]

	Chemical shifts			
	$CDCl_3$	C_5D_5N	C_6D_6	CD_3OD
H-2	2·79	2·97	2·72	2·84
H-3	5·39	5·72	5·87	5·26
H-5	4·70	5·15	5·05	4·66
H-11	5·13	5·68	5·33	5·26
H-13	5·00	5·25	5·15	4·94
	Coupling constants			
	$CDCl_3$	C_5D_5N	C_6D_6	CD_3OD
$J_{2,3}$	11·0	11·0	11·2	11·0
$J_{3,4}$	1·3	1·5	1·0	< 1
$J_{4,5}$	5·5	5·2	6·0	3·9
$J_{10,11}$	1·8	2·0	2·1	1·5
$J_{11,12}$	10·0	9·2	10·0	9·5
$J_{12,13}$	0·8	0·8	0·8	~ 1

[a]Measured from ambient temperature spectra.

the dielectric constant of the solvent, as reflected by changes in the time-averaged coupling constants.[25] The absence of a solvent effect on coupling constants in the erythronolide series is an indication of the presence of a single conformer.

Furthermore, the magnitudes of the coupling constants (both large and small) are extreme values for vicinal couplings (10–12 Hz and 0–2 Hz) and therefore are indicative of a conformationally stable system, since in a conformationally mobile pseudorotating ring system, the coupling constants would be time averaged to intermediate values assuming the various conformers are all significantly populated.[25,26]

In addition, the coupling constants of all derivatives examined are, with three exceptions, remarkably similar. The similarity of the majority of the couplings suggests that the portion of the macrolide ring described by these couplings is conformationally homogeneous throughout the series. Similarly, the correspondence of the chemical shifts of analogous protons in most members of the series examined, supports this contention.

Added support for the proposed relative conformational rigidity of most of the erythromycin derivatives was obtained by carrying out circular dichroism measurements in different solvents. The amplitude of the ketonic $n \rightarrow \pi^*$ Cotton effect is quite sensitive to the nearness of asymmetric centers to the chromophoric grouping, and also to the extent of twisting of the carbon framework in which the CO group is incorporated. Thus, conformational changes can be detected by variations in the intensity of this spectroscopic band. As discussed briefly previously, conformational changes can be induced in flexible molecules by variations in temperature. Such effects can also be induced by alterations in solvent polarity. In extreme cases, solvent induced stabilization of alternative conformations can lead to sign inversions.[27] Another factor which occasionally complicates such studies by contributing to sizeable amplitude alterations is asymmetric solvation.[27] This is most frequently seen with polar solvents and rigid sterically hindered molecules in which the solvent shell in the vicinity of the chromophore is distorted asymmetrically because of molecular crowding. If either asymmetric solvation or conformational flexure were complicating factors in determining the amplitude of the Cotton effects of the erythromycin analogs, then measurement of the spectra in a variety of solvents would surely reveal this. In order to evaluate the effect of non-conformational factors on the system (changes in refractive index, etc) camphor and fenchone were included in the study for comparison. Previous study has shown that these rigid terpenes are relatively free of asymmetric solvational effects.[27] From the data in Table 2 it can be seen that 1 is relatively free of either asymmetric solvation or conformational flexure. This finding is in close agreement with the NMR results. Peak variations of about 10% or less can be accommodated without invoking conformational motion. The data for 1 in isopropanol, dioxane and ethyl acetate are within this range. In methanol, the peak is diminished by about 12% which suggests that some small conformational movement may be taking place.

Table 2. The effect of solvent on the $n \rightarrow \pi^*$ ketone Cotton effect of erythronolide B (1) as compared with camphor and fenchone[a]

Substance	Solvent	$a_{n\rightarrow\pi^*}$	Δ(iPrOH = O)
Erythronolide B (1)	Isopropanol	−170(291 nm)	–
	Dioxane	−184(290 nm)	+8%
	Ethyl Acetate	−175(291 nm)	+3%
	Methanol	−149(290 nm)	−12%
Camphor	Isopropanol	−63	–
	Dioxane	−59	−6%
Fenchone	Isopropanol	−36	–
	Dioxane	−33	−8%

[a]All measurements were made at 30°.

A similar conclusion was reached from chemical and spectroscopic studies with cromycin (13), the unsaturated aglycone of picromycin. Unsuccessful attempts to exchange H-2 in these two compounds were interpreted as evidence of the rigidity of the potentially tautomeric macrocyclic rings. This rigidity was attributed to non-bonded interactions and was reflected by the behavior of C-2 as if it were a bridgehead carbon. This was also apparent in the NMR spectra since there was no significant difference in spectra taken at room temperature and at 160°.[28]

B. *Nuclear magnetic resonance spectra*

1. *Erythronolide B* (1)

The 100 MHz NMR spectrum of erythronolide B (1) in pyridine-d_5 is shown in Fig 1. Individual ring proton and Me group resonances, obtained under high gain and at expanded sweep width are collected in Fig 2.

The chemical shifts and coupling constants determined for this compound are collected in Table 3. These parameters were corroborated by spin decoupling experiments and are generally in agreement with the analysis presented elsewhere by Demarco.[29,30] The coupling constants exhibited by H-12 and H-13, however, have been a source of some ambiguity and these were assigned by spin decoupling experiments performed on a pyridine-d_5 solution of 3,5,11-tribenzoylerythronolide B (14) (Fig 3). The resonance of H-12 is shifted downfield in the spectrum of this compound as a result of deshielding by the adjacent benzoyl group.

When the resonance at 5·98 ppm, assigned to H-11, was irradiated the 3·41 ppm doublet of quartets resonance of H-10 sharpened and the multiplet of H-12 at 2·32 ppm was simplified to a quartet

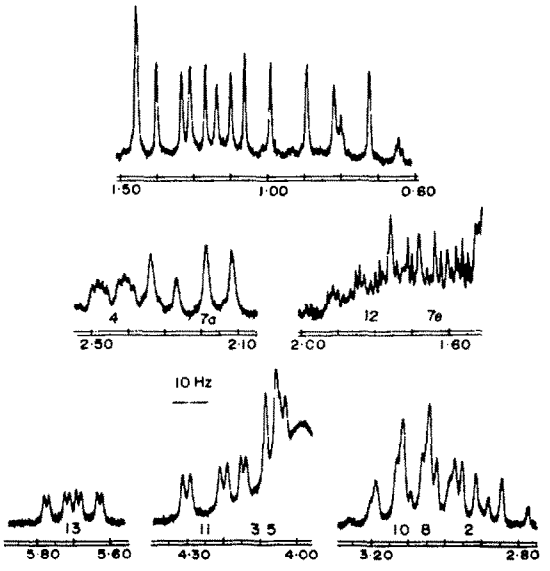


Fig 2. Detail of the spectrum shown in Fig 1. The methyl resonances are recorded at a lower gain than the ring proton resonances.

(Fig 3b). By analogy with erythronolide B (1) these couplings are not unexpected; however, in this case the resonance of H-12 is clearly visible as a five line multiplet consistent with the assignment of a small magnitude to the $J_{12,13}$ coupling.

The resonance of H-13 is a triplet centered at 5·35 ppm partially obscured by the 6-OH group proton resonance. When this resonance was irradiated (Fig 5c), the multiplet assigned to H-12 was only slightly perturbed as evidenced by minor line sharpening. This confirms that in this compound

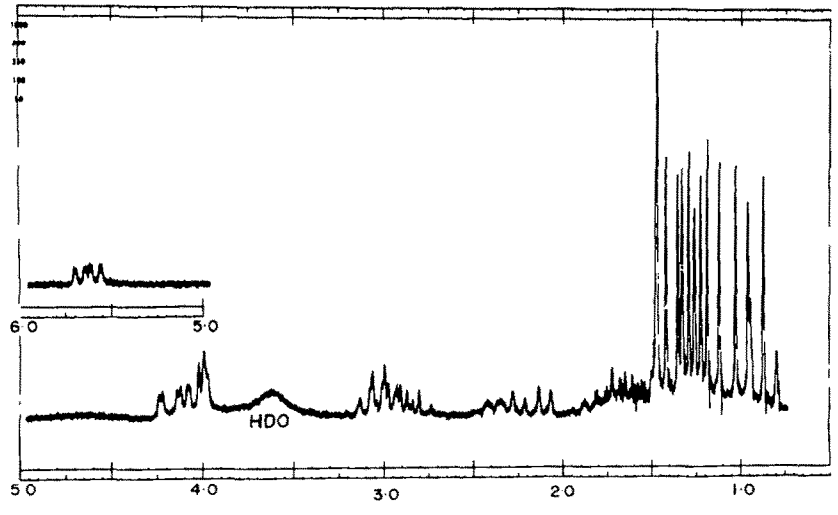


Fig 1. NMR spectrum of erythronolide B (1) in pyridine-d_5 solution at 110° after the addition of sufficient D_2O to exchange hydroxyl protons at 100 MHz.

the coupling of H-13 to the methylene protons is large and $J_{12,13}$ is small (Table 4).

Although not corroborated by spin-decoupling experiments, an analysis of the 220 MHz NMR spectrum of erythronolide B (1) in pyridine-d_5 solution at 110° (Fig 4) confirmed these assignments of the couplings. In this spectrum the H-12 multiplet is clearly resolved at 1·81 ppm as a doublet of doubled quartets (16 lines, the eight most intense of which are clearly visible). In addition, the chemical shifts and geminal coupling constant of H-14a and H-14e can be determined even though their high field resonances are still overlapped.

The spectra of the various acetates of erythronolide B (3–7)[21] were interpreted in a similar manner, and the parameters determined in $CDCl_3$ solution at 55° and pyridine-d_5 solution at 110° are collected with those of 1 in Table 3.

The spectra of the acetates 3–7 revealed a number of changes in the chemical shifts and coupling constant parameters compared to those determined for erythronolide B (1). Downfield shifts of the resonances of H-3, H-5, and/or H-11 were caused by acetylation of the secondary OH groups and were most useful in the structural assignment of these compounds.[21]

Table 3. Chemical shifts and coupling constants of erythronolide B (1) and various acetates 3–7

		Chemical shifts $CDCl_3$				
	1	3	4	5	6	7
H-2	*a*	2·85	2·63	2·75	2·80	2·79
H-3		5·11	3·76	3·96	5·23	5·43
H-4		2·23	2·34	1·72	2·40	2·47
H-5		3·54	4·52	4·04	4·61	4·73
H-7a[c]		1·93	2·05	1·93	2·02	2·17
H-7e		1·42	1·40	1·48	1·38	1·45
H-8		2·74	2·72	3·30	2·74	2·88
H-10		2·93	3·00	2·92	2·99	3·04
H-11		3·83	3·84	4·92	3·83	5·17
H-12		1·70	1·68	1·93	~ 1·7	1·89
H-13		5·44	5·50	5·06	5·50	5·02
H-14a						
H-14e						
		C_5D_5N				
	1[b]	3	4	5	6	7
H-2	2·94	3·02	2·83	2·94	2·96	2·94
H-3	4·08	5·68	4·01	4·24	5·63	5·75
H-4	2·45	2·66	2·73	2·33	2·81	2·78
H-5	4·07	3·86	5·02	4·34	4·99	5·09
H-7a	2·23	2·26	2·37	2·17	2·30	2·34
H-7e	1·64	1·67	1·54	1·69	1·52	1·53
H-8	3·09	~ 3·0	~ 3·0	3·59	~ 3·0	~ 3·1
H-10	3·08	3·14	3·16	3·14	3·15	3·20
H-11	4·25	4·33	4·33	5·27	4·32	5·53
H-12	1·81	1·83	1·85	2·04	1·83	~ 2·0
H-13	5·70	5·81	5·84	5·25	5·82	5·20
H-14a	1·74					
H-14e	1·55					

Table 3 – *Continued*

		Coupling Constants $CDCl_3$				
	1	3	4	5	6	7
$J_{2,3}$	*a*	10·8	10·4	10·0	11·0	10·8
$J_{3,4}$		1·0	< 1	1·0	1·2	1·2
$J_{4,5}$		2·6	3·0	1·8	5·0	5·8
$J_{7a,7e}$		14·5	14·5	14·6	14·7	14·9
$J_{7a,8}$		9·5	10·8	3·2	10·7	6·6
$J_{7e,8}$		3·4	1·6	11·6	2·2	4·2
$J_{10,11}$		1·5	1·2	1·3	1·3	2·0
$J_{11,12}$		9·6	10·0	10·0	9·8	9·8
$J_{12,13}$		1·0	0·8	1·0	0·8	< 1
$J_{13,14a}$		8·8	9·0	8·3	8·8	7·9
$J_{13,14e}$		5·2	5·0	5·7	5·1	6·0
$J_{14a,14e}$						
		C_5D_5N				
	1[b]	3	4	5	6	7
$J_{2,3}$	10·2	10·8	10·0	10·1	10·9	11·0
$J_{3,4}$	1·3	1·5	1·0	1·5	1·4	1·5
$J_{4,5}$	2·9	3·1	3·5	2·0	4·6	5·6
$J_{7a,7e}$	14·5	14·5	14·4	14·5	14·6	15·6
$J_{7a,8}$	6·7	9·2	9·8	3·4	9·4	6·4
$J_{7e,8}$	7·4	4·0	3·2	11·5	3·3	5·5
$J_{10,11}$	2·0	1·8	1·7	1·5	1·5	2·1
$J_{11,12}$	9·8	9·6	10·0	9·6	10·3	9·5
$J_{12,13}$	1·2	1·0	1·2	1·4	0·9	0·9
$J_{13,14a}$	8·8	8·0	8·4	8·0	8·4	8·1
$J_{13,14e}$	6·6	5·5	5·6	5·7	5·6	7·0
$J_{14a,14e}$	14·0					

[a]Erythronolide B (1) is insufficiently soluble in $CDCl_3$.
[b]Measured from 220 MHz spectrum.
[c]The notations 7a, 7e, 14a and 14e are made for convenience. By convention, the low field methylene proton resonance is always denoted as arising from H-7a. These notations do not necessarily reflect the orientation of the protons in the aglycone conformation; however, they were chosen from the orientation in the "diamond lattice" conformation.

The 100 MHz spectrum of 6-deoxyerythronolide B (2) in pyridine-d_5 was not completely interpretable because of severe resonance overlap; however, in $CDCl_3$ solution, sufficient resonances were visible to allow nearly complete analysis. Significant improvement was evident in the 220 MHz spectrum (Fig 5) and complete interpretation became possible. Chemical shift and coupling constant assignments made at 220 MHz and collected in Table 5 were corroborated whenever possible by spin decoupling experiments performed at 100 MHz.

Similarly, the 100 MHz spectra of the various acetate derivatives of 6-deoxyerythronolide B 8–12[21] were obtained in both $CDCl_3$ and pyridine-d_5 solution. Parameters determined from these spectra are collected in Table 5.

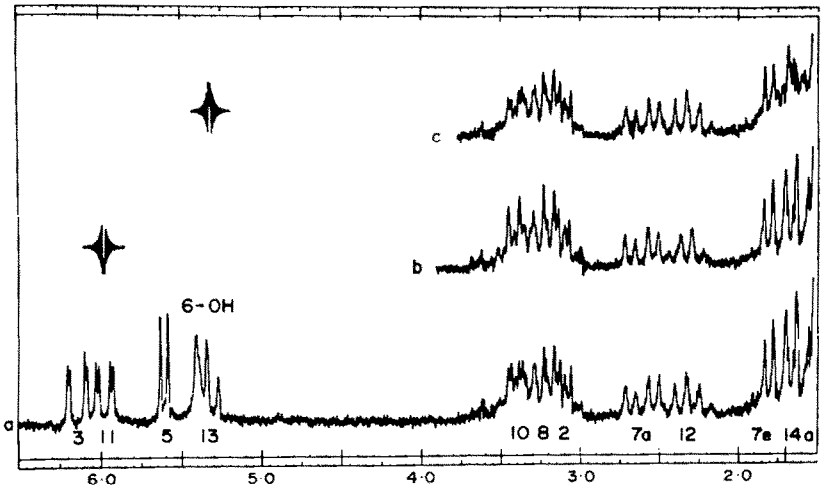


Fig 3. The 100 MHz spectra of 3,5,11-tribenzoylerythronolide B (14) in pyridine-d_5 solution at ambient probe temperature. Scan a: undecoupled spectrum, scan b: spectrum after irradiation of H-11, scan c: spectrum after irradiation of H-13.

Table 4. Chemical shifts and coupling constants of 3,5,11-tribenzoylerythronolide B (14)[a]

	Chemical shifts			Coupling constants	
	$CDCl_3$	C_5D_5N		$CDCl_3$	C_5D_5N
H-2	3·00	3·14	$J_{2,3}$	11·0	10·5
H-3	5·82	6·15	$J_{3,4}$	1·0	1·5
H-4	2·85	3·40	$J_{4,5}$	4·5	4·5
H-5	5·24	5·61	$J_{7a,7e}$	14·8	14·8
H-7a	2·41	2·60	$J_{7a,8}$	7·5	6·5
H-7e	1·62	1·73	$J_{7e,8}$	4·0	5·0
H-8	~ 3·0	~ 3·0	$J_{10,11}$	1·0	2·0
H-10	3·23	3·41	$J_{11,12}$	9·5	9·0
H-11	5·61	5·98	$J_{12,13}$	1·0	1·0
H-12	2·13	2·32	$J_{13,14a}$	7·0	7·5
H-13	5·12	5·35	$J_{13,14e}$	7·0	7·5

[a]Measured from ambient temperature spectrum.

C. *Conformational assignment*

1. *NMR data*

The most useful model compounds for a conformational assignment are 6-deoxyerythronolide B (2) and its acetate derivatives **8–12** since these compounds have vicinal proton substituents at every ring position except those occupied by the ketone and lactone groups, thus providing the most complete experimental data. The known angular dependence of the magnitude of vicinal coupling constants[31–33] can then be used to define the conformation of the ring in these areas.

It has been shown that, while the Karplus relationships are qualitatively valid and the magnitude of coupling constants do vary with dihedral angle, the quantitative value of the coupling also depends on a number of other variables.[34,35] Therefore the Karplus relationships were used in their most general qualitative form.

The orientations of protons in the "diamond lattice" conformation of Dale (Fig 6) are identical

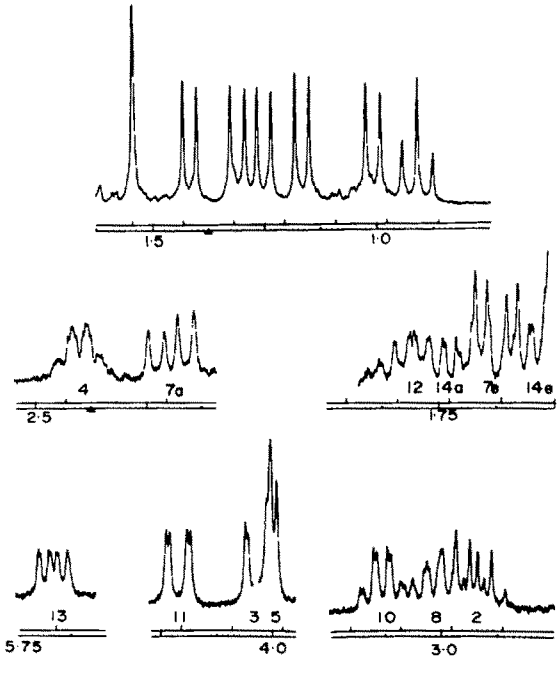


Fig 4. NMR spectrum of erythronolide B (1) at 220 MHz in pyridine-d_5 solution at 110°. Me group resonances are recorded at different gain than the ring proton resonances.

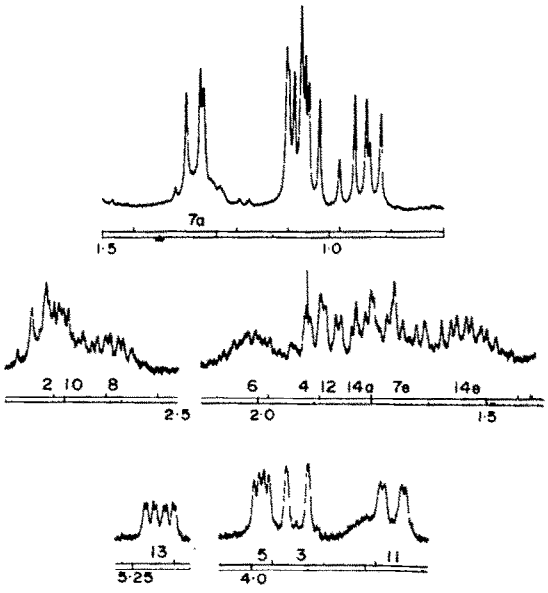


Fig 5. The 220 MHz NMR spectrum of 6-deoxyerythronolide B (2) in $CDCl_3$ solution at 55°. The Me group resonances are recorded at a different gain than the ring proton resonances.

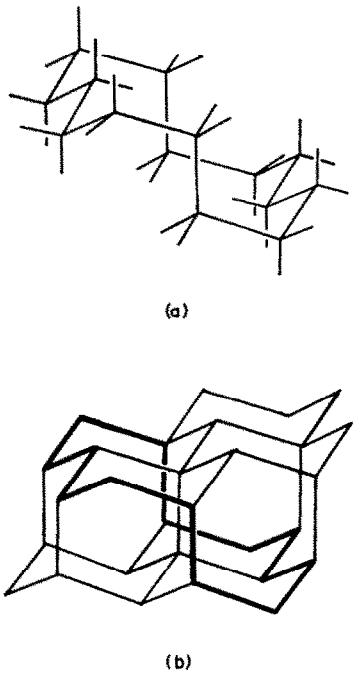


Fig 6. (a) "Diamond Lattice" conformation of cyclotetradecane. (b) Fused 6-membered saturated rings showing the origin of the "diamond-lattice" conformation.

to those in cyclohexane* thereby permitting the differentiation between equatorial and axial protons to be made on the basis of their vicinal coupling constants. In addition, due to inward oriented equatorial protons in this large membered ring, a new vicinal equatorial–equatorial relationship is possible in which the protons are opposed at an angle of 180°. Therefore, the vicinal proton couplings can be segregated into two general ranges in which large couplings, 8–11 Hz, are attributed to equatorial–equatorial (opposed) or diaxial proton orientations, and small couplings, 0–3 Hz, attributed to axial–equatorial or equatorial–equatorial proton orientations.

When a model of 6-deoxyerythronolide B (2) incorporating the configurations required by the configurational model of macrolide antibiotics[36] is placed in the Celmer-Dale conformation (Fig 7), complete agreement between proton relationships and experimental coupling constants is observed (Table 6).[2]

Although the agreement of the NMR parameters with the proposed Celmer-Dale conformation is good, further consideration reveals several discrepancies which indicate that this proposed conformation is neither a favored low energy conformation nor consistent with other physical data.

Study of molecular models of the proposed conformation (Fig 7) shows that an unfavorable 1,3-*syn*-periplanar interaction between the 4- and 6-Me groups is present as is a severe interaction between the 12-methyl and lactone CO group. More serious is the disagreement between the Celmer-Dale conformation and the published single crystal solid state conformation of the aglycone ring of erythromycin A.[37] The shape of a molecule in the crystal state is often a good approximation of its conformation in solution; however, this correlation might be uncertain with potentially flexible structures such as the large ring in erythromycin A. Nevertheless, the X-ray determined conformation of the aglycone ring in the intact antibiotic is such that the 6-OH group, the 9-ketone oxygen and the CO oxygen of the lactone group are all *cis*. The *a*-axis projection[37] of the structure also clearly shows that the C—O bond axes of these three groups are parallel and directed "upward". In the Celmer-Dale conformation, the 6-OH and 9-CO groups are *cis* but their C—O bond axes are directed away from each other and the lactone CO is *trans*.

2. *The Perun conformation*

A critical re-examination of the criterion used in the selection of the most favorable "diamond

*Strictly speaking this is true only for protons situated on the "sides" of the Dale conformation—*i.e.* protons at positions 2–6, 9–13. The protons at the two carbon "bridge" positions—*i.e.*, positions 1, 7, 8, 14 are non-cyclohexanelike; however, they can be given axial and equatorial notations consistent with their relationships to protons at positions 6 and 9, if present.

lattice" conformation disclosed that a second, not previously considered, "diamond lattice" conformation is possible for the aglycone ring.[4,18] The "alternate diamond lattice" conformation is constructed from fused 6-membered rings as in the "original diamond lattice"; however, the carbon atoms are now more nearly planar (Fig 8a). The aglycone ring can be placed into an "alternate diamond lattice" conformation in which the various protons occupy analogous positions (Fig 8b).

It should be emphasized that the "alternate diamond lattice" conformation is not a favorable

Table 5. Chemical shifts and coupling constants of 6-deoxyerythronolide B (2) and various acetates 8–12.

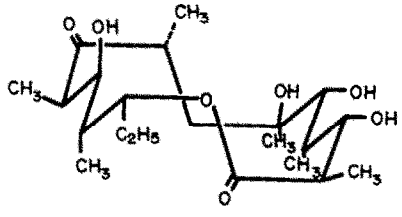
	Chemical shifts $CDCl_3$					
	2[a]	**8**	**9**	**10**	**11**	**12**
H-2	2·78	2·89	2·68	2·72	2·91	2·83
H-3	3·90	5·16	3·71	3·91	5·20	5·23
H-4	1·87	1·86	~ 1·8	~ 2·0	~ 2·0	2·26
H-5	3·98	3·49	4·69	4·04	4·73	4·82
H-6	2·01	~ 1·7	~ 1·7	~ 2·0	~ 2·0	~ 2·1
H-7a	1·25					
H-7e	1·70					
H-8	2·65	~ 2·7	2·56	3·18	2·66	2·82
H-10	2·77	2·78	2·92	2·99	2·81	3·07
H-11	3·69	3·64	3·38	4·93	3·61	4·95
H-12	1·74	~ 1·7	~ 1·7		~ 1·7	~ 2·0
H-13	5·15	5·23	5·37	5·01	5·25	5·00
H-14a	1·82					
H-14e	1·54					
			C_5D_5N			
	2	**8**	**9**	**10**	**11**	**12**
H-2	2·99		2·90	2·91	3·00	2·96
H-3	~ 4·1		3·97	4·16	5·44	5·51
H-4	~ 2·1		2·14	2·25	2·20	2·47
H-5	~ 4·1		5·29	4·23	4·96	5·08
H-6	~ 2·1		~ 1·8	~ 2·1	~ 2·0	~ 2·2
H-7a						
H-7e						
H-8	2·9		2·77	3·35	2·84	2·94
H-10	3·02		3·00	3·14	2·96	3·17
H-11	~ 4·1		3·92	5·24	3·92	5·19
H-12			~ 1·8	~ 1·8	~ 1·8	1·55
H-13	5·59		5·59	5·21	5·61	5·20
H-14a						
H-14e						
	Coupling constants $CDCl_3$					
	2[a]	**8**	**9**	**10**	**11**	**12**
$J_{2,3}$	10·5	10·5	10·2	9·6	10·6	10·6
$J_{3,4}$	< 1	1·5	< 1	1·5	1·2	1·4
$J_{4,5}$	2·5	2·8	4·2	2·2	7·4	6·2
$J_{5,6}$	4·7	5·2	2·3	4·5	1·8	2·0
$J_{6,7a}$	4·7					
$J_{6,7e}$	10·2					
$J_{7a,7e}$	15·0					
$J_{7a,8}$	13·0			12·0	9·0	6·5
$J_{7e,8}$	4·0			4·5	5·5	6·5
$J_{10,11}$	2·0	2·0	1·5	1·3	2·0	1·5
$J_{11,12}$	10·2	9·8	9·8	10·0	9·8	10·0
$J_{12,13}$	1·5	1·3	1·5	1·2	1·5	1·3
$J_{13,14a}$	8·9	8·8	9·0	8·2	8·8	8·0
$J_{13,14e}$	4·6	4·8	5·0	6·0	4·8	6·0
$J_{14a,14e}$	14·0					

Table 5—*Continued*

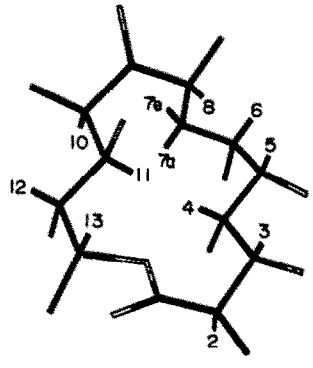
	Coupling constants C_5D_5N					
	2	**8**	**9**	**10**	**11**	**12**
$J_{2,3}$	10·0		9·5	9·6	10·4	10·4
$J_{3,4}$			1·2	1·6	1·2	1·6
$J_{4,5}$			6·2	3·5	7·0	6·3
$J_{5,6}$			2·2	3·5	2·0	2·0
$J_{6,7a}$						
$J_{6,7e}$						
$J_{7a,7e}$						
$J_{7a,8}$				10·2	9·5	6·5
$J_{7e,8}$				4·0	4·5	6·5
$J_{10,11}$	2·0		1·9	1·4	2·0	1·6
$J_{11,12}$			9·5	9·6	9·8	9·8
$J_{12,13}$	1·5		1·2	1·2	1·2	1·5
$J_{13,14a}$	8·6		8·5	8·2	8·5	7·6
$J_{13,14e}$	5·2		5·5	6·0	5·4	6·0
$J_{14a,14e}$						

[a]Measured from 220 MHz spectrum.

low energy conformation for the cyclic hydrocarbon or for the aglycone ring and was therefore not considered by Dale. In the 14-membered ring hydrocarbon, intraannular interactions between the four inward pointing substituents are prohibitive. The instability of this conformation for the aglycone is a result of these same intraannular interactions between H-4 and 8-CH_3 (Fig 8b) although they have been reduced somewhat by incorporation of the lactone which reduces the number of inward directed protons. In addition, 1,3-*syn*-periplanar interactions between the 4- and 6-Me groups destabilize this aglycone conformation (Fig 8b). Nevertheless, the "alternate diamond lattice" is a useful model for the final conformation of the aglycone ring since the lactone and ketone CO groups are *cis*, and the instabilities can be reduced by conformational reorganizations which also correctly orient other substituents.



(a)



(b)

Fig 7. The Celmer-Dale conformation of 6-deoxyerythronolide B (2). (a) Projection showing the orientations of substituents. (b) Photograph of Framework Molecular Model construction. 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 6. Proposed vicinal proton orientations from the Celmer-Dale conformation and experimental coupling constants for 6-deoxyerythronolide B (2) in $CDCl_3$ solution

Interacting protons	Orientations[a]	Coupling[c]
$J_{2,3}$	axial–axial	10·5
$J_{3,4}$	axial–equatorial	< 1
$J_{4,5}$	equatorial–axial	2·5
$J_{5,6}$	axial–equatorial	4·7
$J_{6,7a}$	dihedral angle 60°	4·7
$J_{6,7e}$	dihedral angle 180°	10·2
$J_{7a,8}$	dihedral angle 180°	13·0
$J_{7e,8}$	dihedral angle 60°	4·0
$J_{10,11}$	axial–equatorial	2·0
$J_{11,12}$	equatorial–equatorial[b]	10·2
$J_{12,13}$	equatorial–axial	1·5

[a]Since substituents on C-7 and C-8 are on non-cyclohexanelike carbon atoms, dihedral angles rather than axial–equatorial relationships are given.

[b]Dihedral angle 180°.

[c]Measured from 220 MHz spectrum.

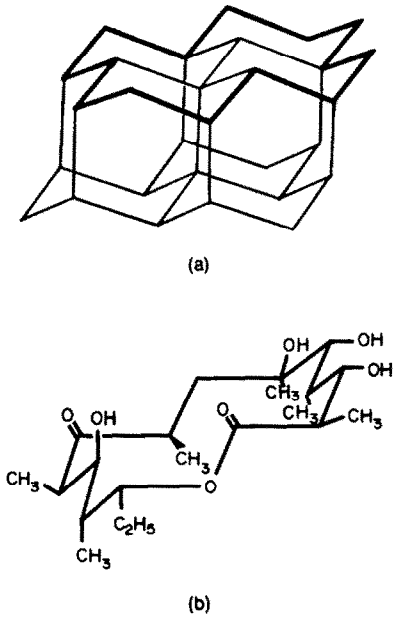


Fig 8. The alternate "diamond lattice" conformation. (a) Fused 6-membered saturated rings showing the origin of the alternate "diamond lattice". (b) The alternate "diamond lattice" conformation of erythronolide B showing the relationship of substituents.

As in the Celmer-Dale conformation, the "alternate diamond lattice" conformation places the 6-OH group *cis* to the ketone CO, but the C—O bond axes are not oriented in the same direction. However, simultaneous rotation of the 5–6 and 7–8 bonds of the "alternate diamond lattice" conformation in such a manner that the 6-OH group is displaced "up" and toward the center of the ring while the 7-methylene group is rotated "downwards" and away from the center of the ring places these groups in the proper orientation. This final conformation (the Perun conformation) (Fig 9) incorporates relative positions of the ring protons and those of the chromophoric lactone and ketone groups and the 6-hydroxyl group which satisfy both the X-ray and NMR data.

3. *CD data*

Having presented data in earlier paragraphs which argue strongly against either asymmetric solvation or conformational flexure playing a significant role in determining peak amplitudes for the ketone Cotton effect of erythromycin derivatives, it is apparent that the amplitudes observed must reflect the molecular conformation in the vicinity of the chromophoric regions of the molecule. This is particularly fortunate, because lack of H atoms in these functions render these "blind" spots in which the conformation cannot be easily settled by NMR measurements. In addition, the ketone and lactone

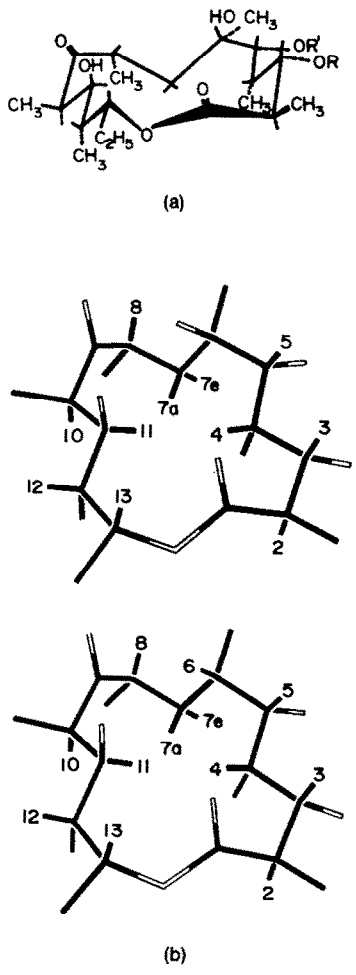


Fig 9. The Perun conformation of erythronolide B (1). (a) The Perun conformation showing the relationship between substituents. (b) Photographs of models of erythronolide B and 6-deoxyerythronolide B showing the relationship between protons.

functions are widely separated in the molecule and their energy content is quite different; thus, the two CD bands should be relatively independent of one another so that curve factoring can be done with confidence. Because each chromophore is sensitive to conformational changes in its immediate environment, CD measurements should allow one not only to choose between alternate conformational possibilities, but also to detect conformational alterations due to substituent alterations.

The typical erythronolide analog shows a negative Cotton effect at about 290 nm due to the ketone and a negative peak at about 215 nm due to the lactone moiety (Table 7). The ketone transition can be analyzed by use of the well-known octant rule.[38] The octant projection for the Perun conformation is given in Fig 10a. The Me group at C-8 is axial and

Table 7. CD spectra of selected erythronolide B derivatives in methanol solvent

	Ketone		Lactone	
	nm	[θ]	nm	[θ]
Erythronolide B (1)	290	−12180	210	−4260
6-Deoxyerythronolide B (2)	290	−17482	215	−5420
3-Acetylerythronolide B (3)	290	−11626		–
11-Acetylerythronolide B (5)	292	−17875	218	−3200
3,5-Diacetylerythronolide B (6)	292	−11103	216	+3170
3-Acetyl-6-deoxyerythronolide B (8)	289	−17128	220	−2320
5-Acetyl-6-deoxyerythronolide B (9)	289	−16371	212	−5300
11-Acetyl-6-deoxyerythronolide B (10)	290	−17400	222	−3815
3,5-Diacetyl-6-deoxyerythronolide (11)	291	−16260	230	−1305
Erythronolide B 3,5-phenylboronate (15)	292	−15690		–
6-Deoxyerythronolide B 3,5-phenylboronate (16)	290	−20625		–

rather close to the ketone in a negative octant. The methyl group at C-10 is equatorial and nearly nodal so that its influence on the ketone amplitude should be minimal. The OH group at C-6 is quite close to the ketone function and is in a positive octant. The OH group at C-11 is rather close to the vertical nodal plane, but is present in a negative octant rather close to the ketone. The overall prediction from these considerations is a negative peak, in agreement with the experimental findings. The amplitude is somewhat higher than normal for a cyclohexanone suggesting that the chromophore is somewhat twisted in a negative helical sense.[39]

It is possible to support these conclusions by measuring the spectra of some derivatives in which key functional groups in the immediate vicinity of the chromophore have been altered but whose NMR spectra indicate that this change has not much affected the molecular conformation. For example, when the 6-OH group is absent, as in 6-deoxyerythronolide B (2), the negative amplitude of the ketone transition is augmented as would be expected for removal of a positive sign contributing function from the vicinity of the chromophore. The Celmer-Dale conformation is not consistent with these findings, for the C-6 OH group is not only distant from the ketone, but is actually in a negative octant (Fig 10b).

The negative octant location of the C-11 OH group was at first thought to be confirmed by the strong increase in the negative intensity of the ketone Cotton effect of 3,5,11-triacetylerythronolide B (7) compared with that of erythronolide B (1) itself.[5] Detailed NMR examination of these two compounds described in a forthcoming paper has shown them to possess somewhat different conformations. Detailed studies indicate that the C-11 function lies rather close to the ketone nodal space and increased bulk, as with acetylation triggers a conformational movement which causes some derivatives to show no amplitude change on C-11 acetylation, others to grow more negative, and yet others to show a positive contribution to the ketone band.

The best way to rationalize the signs and amplitudes of lactones is still under active discussion. The Klyne lactone sector rule[40] works rather well, but sufficient exceptions exist to encourage others to propose alternatives. Snatzke has proposed a modified sector rule[41] which allows for more CO character for the lactone CO than does the Klyne treatment. Beecham and Wolf have proposed a quite different rule[42] which treats the lactone as an inherently twisted system and, thus, rationalizes some difficult cases. One must point out at the outset that there are no precedent cases in the litera-

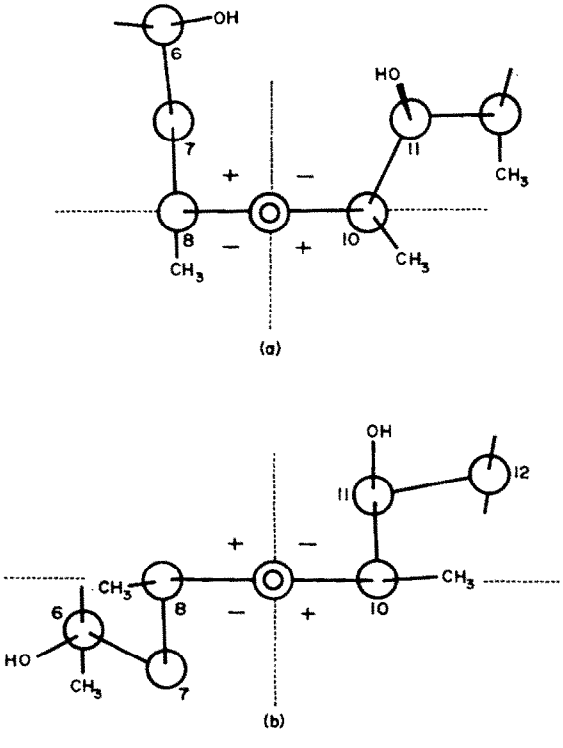


Fig 10. The ketone octant projections of the various conformations of erythronolide B (1). (a) The Perun conformation. (b) The Celmer-Dale conformation.

ture involving lactone rings as large as those seen with the macrolide antibiotics. Nevertheless, there is no reason to suspect that the electronics of excitation will be in any way exceptional for these compounds, and one expects that the rules will be applicable. Fortunately, all three predictive rules require a negative peak for the lactone Cotton effect in the erythromycin derivatives in the Perun conformation. This not only agrees with the results of the X-ray study, but also allows the lactone to be in the energetically most favored S-*anti* conformation.[43] In the Beecham-Wolf treatment (Fig 11a) a negative peak is predicted as C-3 lies below the plane of the chromophore and there are more uncompensated atoms on that side of the molecule in the vicinity of the chromophore. The Klyne and Snatzke treatments are difficult to diagram in two dimensions for the erythromycin macrolides because the majority of the atoms in the molecule lie at right angles to the chromophoric plane and thus overlie one another in drawings. It is interesting to note that some significant alterations take place in lactone amplitude when substituent changes are made at C-3 or C-5. Because these groups are rather close to the chromophore, and are on the sign determining atom in the Beecham treatment, this would be expected. The C-3 oxygen substituent lies fairly close to the chromophoric plane and it would not require a major conformational alteration to cause this grouping to pass over into an oppositely signed sector. Acetylation of the hydroxyl groups on either C-3 or C-5 would interrupt H-bonding between these two groups and interrupt the colinearity of these functions. This would be transmitted to the chromophore and appear in the CD spectrum. Indeed, this effect is also clear in the NMR spectrum.

The Celmer-Dale model (Fig 11b) would lead to *positive* peak prediction based upon chirality rules as well as lactone sector rules. In the latter, the significant atoms are predominantly in positive sectors.

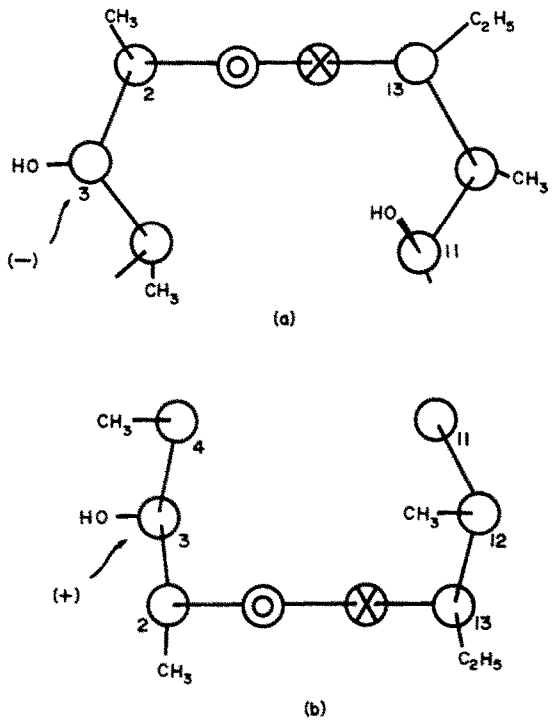
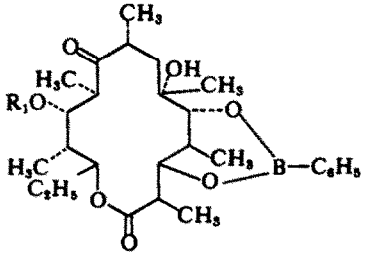


Fig 11. The Beecham-Wolf lactone sector projections of the various conformations of erythronolide B (**1**). (a) The Perun conformation. (b) The Celmer-Dale conformation.

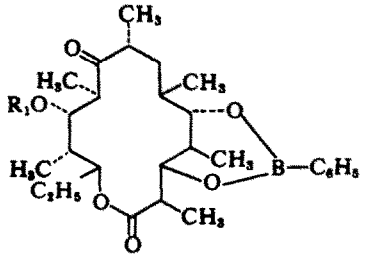
4. *Confirmatory evidence*

a. Syn-*periplanar hydroxyl groups at* C-3 *and* C-5. Coupling constant data suggest that the equatorial 3- and 5-OH groups are *syn*-periplanar. This is not unexpected in view of recent findings with *meso*-2,4-pentanediol which revealed that the intramolecular H-bond, possible only between *syn*-periplanar OH groups, offers sufficient stabilization so that conformations incorporating this relationship predominate in non-polar solvents.[44] Examination of the NMR spectra of the 3,5-phenylboronate[21] of erythronolide B (**15**) and 6-deoxyerythronolide B (**16**)[18] and their 11-acetates (**17**, **18**)[21] offered evidence for this suggestion. The 6-membered ring geometry of the phenylboronate group requires that the esterified hydroxyls be *syn*-periplanar.

Complete analysis of the spectra of these compounds was not possible due to their instability and the extensive line broadening that was evident



15: R = H
17: R = Ac



16: R = H
18: R = Ac

when the spectra were recorded at elevated temperatures. The latter effect is most probably either a result of the quadrupole moment of the boron nuclei or slow boron tetrahedral inversion and not a result of conformational changes in the aglycone ring. Nevertheless, when the coupling constants of the phenylboronates (Table 8) and the non-esterified aglycones (Tables 3 and 5) are compared, no significant changes in the magnitude are observed. This confirms that no change in the relative orientations of the OH groups was required to form the 6-membered boronate, and therefore, the 3- and 5-hydroxyl groups are *syn*-periplanar in 1 and 2.

Table 8. Chemical shifts and coupling constants of erythronolide B 3,5-phenylboronate (15), 6-deoxyerythronolide B 3,5-phenylboronate (16), and their 11-acetates 17, 18

	Chemical shifts							
		$CDCl_3$			C_5D_5N			
	15[a,b]	16	17[a]	18[a]	15[c]	16[b]	17	18
H-2	2·8	2·90	2·84	2·83	3·0		2·91	2·92
H-3	4·26	4·20	4·28	4·22	4·33		4·35	4·31
H-4		2·5	2·62	2·64			2·77	2·79
H-5	4·47	4·42	4·59	4·52	4·65		4·74	4·59
H-6	–	2·5	–	2·48	–		–	2·46
H-8	2·8	2·7	3·41	3·27	3·0		3·62	3·41
H-10	2·8	2·84	2·89	3·02	3·0		3·04	3·12
H-11	3·89	3·97	5·14	5·24	4·2		5·34	5·40
H-12		1·9	1·91					1·98
H-13	5·35	5·35	5·08	5·00	5·8		5·21	5·14

	Coupling constants							
	15	16	17	18	15	16	17	18
$J_{2,3}$	10·5	10·6	11·0	11·0	10·0		10·0	11·0
$J_{3,4}$	1·2	1·5	2·0	1·5	2·0		2·0	2·0
$J_{4,5}$	2·0	2·2	2·0	2·0	2		2	2·0
$J_{5,6}$	–	6·5	–	6·2	–		–	6·0
$J_{10,11}$		1·5	3·0	1·8			2·8	1·8
$J_{11,12}$		10·0	10·0	10·1			10·0	10·1
$J_{12,13}$	1·2	1·2	1	1			< 1	1·1
$J_{13,14a}$	9·0	8·6	7·0	7·0			7·0	6·2
$J_{13,14e}$	4·7	4·8	7·0	7·0			7·0	6·2

[a]Data measured from ambient temperature spectrum.
[b]Sample unstable.
[c]Extensive line broadening observed.

b. *Relative orientation of the* 11*-hydroxyl group.* The orientation of the 11-OH group in 1 and 2 was confirmed by consideration of the effect of the acetylation of this OH group on the chemical shifts of protons on adjacent positions. Although the deshielding effect produced by esterifying a OH group on the proton or protons on the carbon holding the OH group is well documented,[45] only a few examples of the effect on protons at adjacent positions have been published.[46-48] The effect is known to be most pronounced when the OH group is axial[46] and is also recognized as being dependent on the relative orientation of the CO group of the ester to the adjacent protons.[46,48] The largest effects were anticipated to be associated with acetylation of the 11-OH group since this is the only axial OH group.

The large upfield shift of H-13 when the 11-OH group is acetylated in either the monoacetyl or triacetyl derivatives of both erythronolide B or 6-deoxyerythronolide B is a significant one (Tables 3 and 5). The magnitude and direction of the shift, between +0·40 and +0·50 ppm,* is consistent with the shifts previously reported for protons *syn*-periplanar to an acetylated OH group.[46,48] Since H-13 and the 11-OH group are not on adjacent carbons and since the effect is significant, H-13 and the 11-OH group must be *syn*-periplanar. The marked upfield shift of the resonance of H-13 on acetylation of the 11-OH group has been shown to be a general phenomenon[20,49] and is diagnostic for an 11-OH group.

These data combined with that collected from the CD spectra are totally complementary and define in detail the solution conformation of erythronolide B and simple derivatives. The data reveal that the conformation is totally staggered and incorporates no destabilizing eclipsed groups or *syn*-periplanar arrangements. Most importantly, the vicinal proton relationships which are required by the magnitude of the vicinal coupling constants previously determined (Tables 3 and 5) are correctly incorporated without exception. Therefore, the Perun conformation (Fig 9) satisfies all the physical data collected on the aglycone to date and incorporates no arrangements of groups which are expected to be unfavorable.

Acknowledgements—The authors wish to thank P. W. Howison† and B. Slater Eng of the Ohio State University for measuring the CD spectra and L. W. Cary of Varian Associates for the 220 MHz NMR spectra. Many helpful discussions with Dr. M. I. Levenberg, Dr. J. S. Tadanier, and Dr. P. H. Jones of Abbott Laboratories and Dr. P. A. Beak of The University of Illinois are gratefully acknowledged.

*Positive values represent upfield shifts (shielding).

†N.S.F. Undergraduate Research Participant, 1970 under grant number GY7446.

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