Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Some Macrolide Antibiotics and Derivatives. Substituent and Conformational Effects[1a]

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Abstract: Carbon-13 (^{13}C NMR) chemical shifts are reported and assigned for a series of naturally occurring macrolide antibiotics, biosynthetic precursors, shunt metabolites, and derivatives. Besides the usual ^{13}C NMR substituent effects, the results are interpreted with reference to conformational models proposed for these compounds. A number of proposals are made concerning the conformations of the various derivatives, the differences between them, and the possibility of internal motion. The usefulness of ^{13}C NMR in ascertaining hydrogen bonding and in the structural analysis of these important compounds is demonstrated.

Carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR) of natural products is recognized as a useful tool in problems of structural and conformational analysis.[2] In the present study, we report the ^{13}C NMR spectra of a series of macrolide antibiotics and derivatives and interpret these data from the standpoint of conformational considerations as well as previously established effects on ^{13}C NMR chemical shifts. These are complex substances, and matters are made worse by the fact that macrolide ^{13}C NMR spectra are expected to involve special problems with regard to known substituent and steric effects. Particularly vexing is the fact that the effect of adding or removing a substituent should be smaller than in most of the model systems because of the observed attenuation of substituent effects with increasing substitution.[2,3] Furthermore, there are likely to be important conformational changes with substitution which will make the β effect smaller than usual and the γ effect variable. Thus, when a substituent is removed, the expected upfield (negative) β shift can be opposed by a coincident downfield shift caused by conformational reorganization which reduces steric compression. Thus, a β effect, already reduced by extensive substitution, could be even further reduced. On top of this, steric shifts are expected to be much harder to predict because of the many possible steric interactions around and inside the large ring. For this reason, especially heavy reliance has had to be put on single frequency decoupling (SFD) and/or off-resonance decoupling (ORD) data.

Experimental Section

All of the spectra were taken on the previously described Varian DFS-60[4] (subsequently modified to a pulsed Fourier transform), PFT (Brukarian[5]) or HR-220 spectrometer. Most of the 15-MHz spectra and all of the 55-MHz spectra were obtained with a pulsed Fourier transform system. Unless otherwise noted, spectra were of solutions in 4:1 (v/v) mixtures of $CDCl_3$ and CH_2Cl_2. Chemical shifts were measured relative to internal CH_2Cl_2 and converted to internal CS_2 using the relationship: $\delta(CS_2) = \delta(CH_2Cl_2) + 138.8$. The resulting values can be converted to the currently favored internal Me_4Si scale by $\delta(Me_4Si) = 192.8 - \delta(CS_2)$. The estimated errors in the chemical shift, unless otherwise noted, are ± 0.1 ppm. Typical spectra used 0.5–1.0 ml of solvent at the ambient probe temperature (30–35°) with proton noise, off-resonance, or single-frequency decoupling (SFD). The deuterium signal from $CDCl_3$ was used as a field-frequency lock. Spectra could usually be obtained at concentrations greater than 0.1 *M*, but concentrations of 0.3–0.4 *M* were more usual.

Results

1. The Aglycons. To begin with, the ^{13}C NMR spectra were taken and assigned for nine macrolide aglycon derivatives. An example of a proton noise-decoupled spectrum for 6-deoxyerythronolide B (**1**) is shown in Figure 1. This spectrum is for an approximately 0.8 *M* solution, taken in less than 30 min on the DFS-60 spectrometer. Assignments for the spectra of these nine derivatives plus the hemiacetal of 5-deoxy-5-oxoerythronolide B (which is in equilibrium with the keto form) are given in Tables I–III. Ambiguous assignments are indicated on the tables; in each case, the tabulated assignment is considered to be more probable. Discussion of how assignments were made will proceed by region of the spectrum. The letter A, followed by a number, refers to "aglycon, position—". Later, the letter D will be used in the same way for desosamine, the letter C for cladinose, the letter M for mycarose, the letter O for oleandrose, the letter L for lankavose, and the letter R for rhodosamine. Because of potential difficulties in keeping straight the names and numbers of the players in the complex sequel, the names, structures, and reference numbers of all of the substances are given in Figures 2–6.

The oxygenated carbons are the ketone and lactone carbonyls A9 and A1, the three secondary alcohol carbons A3, A5, A11, the tertiary alcohol carbon A6, and the lactone ester A13. The assignment of A9 was generally straightforward, because ketone carbonyls appear at lower field than any other type of carbon which needs to be considered here. Only in the case of the 5-oxo derivatives was ambiguity possible. Differentiation in this case could be made by the observation of a (negative) β shift in going from 5-deoxy-5-oxoerythronolide B (**3a**) to 5,6-dideoxy-5-oxoerythronolide B (**4**) and the comparison of **3a** with its hemiketal **3b**. Assignment of A1 was also usually easy. The only possible ambiguities here were with the acetoxy derivatives; however, the acetoxy carbonyls are generally 3–6 ppm upfield from the lactone carbonyls.

Most of the assignments of A3, A5, A6, A11, and A13 were made by single-frequency decoupling (SFD) and off-resonance decoupling (ORD). Here, great help was provided by the extensive proton-shift data summarized by Egan.[6] Unequivocal SFD assignments could usually be made if the proton signals differed by more than 0.1 ppm. The spectrum of erythronolide B (**2**) was taken on a very dilute sample in dioxane, because it is nearly insoluble in deuteriochloro-

Table I. Aglycon ^{13}C NMR Shifts and Assignments for Oxygenated Carbons (ppm from CS_2)

Carbon	1	2[a]	3a	3b	4[c]	5	6	7	8	9
A9	−22.1	*b*	−23.7	81.2 ± 1	−23.0	−20.5 ± 1.5	−20.7	−27.2	−22.5	110.9
A1	14.1	14.8 ± 1	16.8	Ca. 15	16.8	17.2	17.4	17.9	19.5	14.1
A3	113.2	113.4	120.5	121.2	120.9	112.7[d]	113.6	115.1	115.9	112.1[d]
A5	116.3	111.5	−26.5	Ca. −25	−25.0	113.0[d]	116.4	113.1	113.7	112.4[d]
A6	157.2	117.8	113.4	105.9	147.2	116.6	156.9	118.0	118.4	116.5
A11	121.6	122.6	122.8	122.4	122.0	117.6	118.2	122.7	119.9	121.8
A13	116.3	117.8	116.5	116.0	117.1	118.0	118.2	117.2	118.4	116.5

[a] Dioxane as solvent. [b] Not investigated. [c] CH_2Cl_2 as solvent. [d] Interchangeable assignment within the same vertical column of the table.

Table II. Aglycon ^{13}C NMR Shifts and Assignments for Methine and Methylene Carbons (ppm from CS_2)

	1	2[a]	3a	3b	4[c]	5	6	7	8	9
A6	157.2[b]	117.8	113.4	105.9	147.2	116.6	156.9	118.0	118.4	116.5
A2	149.1	148.8	148.9	148.2	148.9	149.2[b]	149.4[b]	149.9	149.7[d]	148.2[b]
A8	152.8[d]	150.6[b]	147.0	141.0	145.9	149.9[b]	152.5[b]	147.1	150.3[d]	158.2
A10	148.6[d]	153.1[b]	154.8[b]	155.1	153.4[b]	153.4[b]	150.2[b]	153.8[b]	152.5[b]	160.5
A4	155.0[b]	156.4	151.4[b]	149.2	145.9	155.8[b]	155.2[b]	156.6[b]	156.5[b]	156.3
A12	152.0[b]	152.2	151.9[b]	*e*	152.2[b]	153.4[b]	153.5[b]	152.2[b]	153.4[b]	152.2
A7	155.0[b]	151.6	151.9[b]	150.2	156.0[b]	150.6[b]	155.2[b]	155.0[b]	155.8[b]	151.4

[a] Dioxane as solvent. [b] Confirmed by SFD. [c] CH_2Cl_2 as solvent. [d] Interchangeable with others in same vertical column, most likely assignments are as shown. [e] Not detected or overlapped.

Table III. Aglycon ^{13}C NMR Shifts and Assignments for Methyl Carbons (ppm from CS_2)

	1	2[a]	3a	3b	4[c]	5	6	7	8	9
A14	167.2	166.7[d]	166.6	167.2	167.0	166.5[d]	166.6	166.4	166.4[d]	167.3
6-Me	176.0	167.0[d]	171.3	167.7	176.4	165.9[d]	175.7	166.4	166.8[d]	165.9
12-Me	183.6	183.9	183.6	184.3	183.7[d]	182.8	182.8	183.5[d]	182.9	184.1
4-Me	185.8[d]	186.2	182.8[d]	*b*	183.7[d]	186.1	185.1	183.5[d]	183.7[e]	187.2
10-Me	179.4[e]	176.3[e]	174.8	*b*	178.4	176.7[e]	178.5	177.5	178.0	175.4
2-Me	178.0[e]	178.1[e]	178.4	177.6	178.4	178.1[e]	178.5	183.0[d]	182.4[e]	178.2
8-Me	186.5[d]	185.0	183.1[d]	*b*	183.9[d]	184.8	185.1	183.5[d]	183.7[e]	182.7
A15	182.1	182.7	182.3	*b*	182.4	182.1	182.2	181.9	182.4	182.2
Ac-Me						171.8	171.5	171.5 (A3) 173.8 (A5)	171.7 All 3	

[a] Dioxane as solvent. [b] Not detected or overlapped. [c] CH_2Cl_2 as solvent. [d,e] Interchangeable with others of the same vertical column of the table.

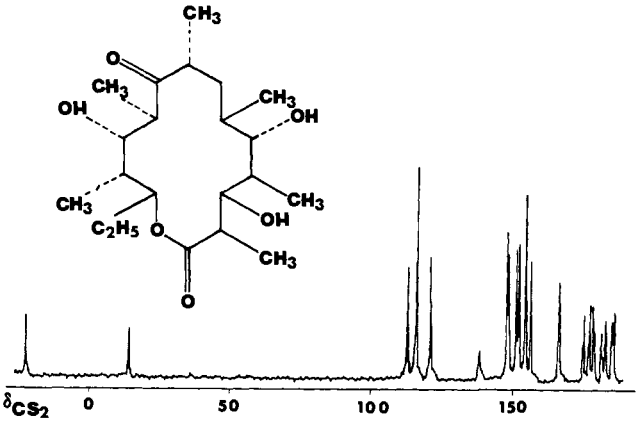
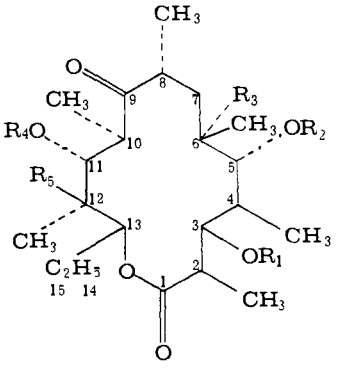


Figure 1. Proton noise-decoupled ^{13}C NMR spectrum of 6-deoxyerythronolide B.

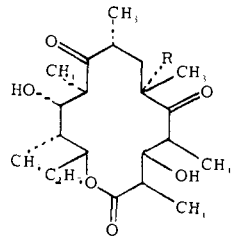


		R_1	R_2	R_3	R_4	R_5
1	6-deoxyerythronolide B	H	H	H	H	H
2	erythronolide B	H	H	OH	H	H
5	11-acetylerythronolide B	H	H	OH	Ac	H
6	11-acetyl-6-deoxyerythronolide B	H	H	H	Ac	H
7	3,5-diacetylerythronolide B	Ac	Ac	OH	H	H
8	3,5,11-triacetylerythronolide B	Ac	Ac	OH	Ac	H
12	3-O-α-L-mycarosylerythronolide B	(*27*)	H	OH	H	H
13	5-O-β-D-desosaminylerythronolide B	H	(*11*)	OH	H	H
14	erythromycin A	(*10*)	(*11*)	OH	H	OH
15	erythromycin B	(*10*)	(*11*)	OH	H	H
16	de-N-methylaminoerythromycin A	(*10*)	(*28*)	OH	H	OH
17	de-N-methylaminoerythromycin B	(*10*)	(*28*)	OH	H	H
18	3'-dedimethylamino-3',4'-dehydroerythromycin A	(*10*)	(*33*)	OH	H	OH
26	megalomicin A	(*27*)	(*11*)	OH	(*29*)	OH

Figure 2.

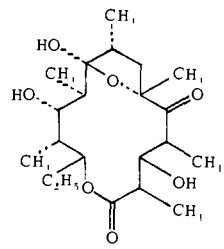
form. The shifts of 5,6-dideoxy-5-oxoerythronolide B (**4**) are reported for methylene chloride, but little solvent shift was observed for comparison spectra taken in $CDCl_3$–CH_2Cl_2 mixtures. The resonances in the spectrum of the hemiketal of 5-deoxy-5-oxoerythronolide B (**3b**) were not all found in this relatively insoluble sample. Assignments were made by comparison with the ketone spectrum and with the other ketal spectra. The changes observed on going from the keto to hemiketal form will be discussed later.

The remaining aglycon ring carbons A2, A8, A10, A4, A12, and A7 proved to be the most difficult to assign unambiguously. For this region, we have noted in Table II the certain assignments based on SFD experiments. The requisite proton shifts were available for most cases, but problems arose because of the complexity of the proton spectra. In all cases where the proton shifts could be used, the peaks representing A2, A8, and A10 were distinguished from

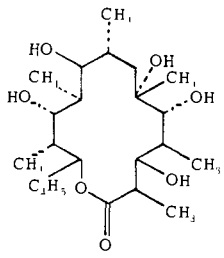


3a 5-deoxy-5-oxoerythronolide B R=OH

4 5,6-dideoxy-5-oxoerylhronolide B R H

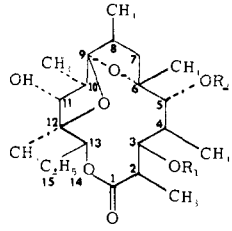


3b 5-deoxy-5-oxoerythronolide B-6,9-hemiketal

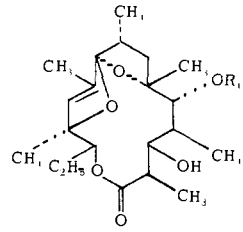


9 9S-9-dihydroerythronolide B

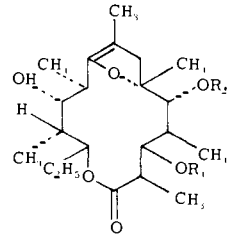
Figure 3.



19 anhydroerythromycin A:
R_1 α-L-cladinose 10;
R_2 β-D-desosamine 11



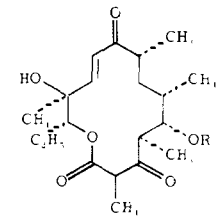
20 erythralosamine:
R_1 = β-D-desosamine 11



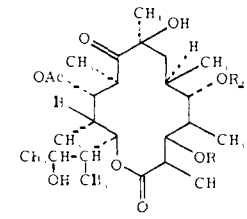
21 8,9 anhydroerythromycin B-6,9-hemiketal:
R_1 = α-L-cladinose 10;
R_2 = β-D-desosamine 11

22 8,9 anhydroerythronolide B-6,9-hemiketal:
R_1 = H, R_2 = H

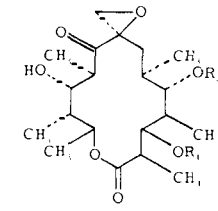
Figure 4.



23 picromycin:
R = 3-D-desosamine 11



24 lankamycin: R_1 α-4-O-acetyl-L-arcanose 30,
R_2 β-D-lankavose 31



25 oleandomycin, R_1 = L-oleandrose 32;
R_2 = D-desosamine 11

Figure 5.

those representing A4, A12, and A7. Further, A7 could often be definitely assigned by ORD or SFD, because it is methylene rather than a methine. The spectra of 11-acetylerythronolide B (**5**) and 11-acetyl-6-deoxyerythronolide B (**6**) could be completely assigned in this region by SFD. Beyond this, some comparative assignments had to be made. A2 was generally assigned by SFD, but for those not so indicated in Table II, the A2 assignments were based on the fact that the macrolide conformations appear similar at this carbon for all derivatives without a substituent on the adjacent (A3) hydroxy group, as judged by Egan from his study[6] of vicinal proton couplings. In the 3-acetoxy derivatives, an upfield shift would be expected because of steric interaction with the freely rotating acetoxy group.

The assignments of A8 and A10 were difficult. Those for 6-deoxyerythronolide B (**1**) were inferred from repeated SFD experiments. Here, the proton signals differ by 0.12 ppm.[6] For (9*S*)-9-dihydroerythronolide B (**9**), no SFD was possible, and the assignments of A8 and A10 depend on the observed upfield shift for the immediately adjacent carbons as the result of reducing a ketone to an alcohol. This is one substituent effect which was consistently observed at near its expected value in the entire series. The assignment of A8 in 3,5-diacetylerythronolide B (**7**) is made by comparison with 3,5,11-triacetylerythronolide B (**8**). The proton frequencies of A8 and A2 were too close for **7** to use SFD. Egan's results[6] show that the only differences in the vicinal proton couplings between these two compounds are in the A8 region. The assignments of A4, A12, and A7 are almost all unequivocal by SFD. Only for (9*S*)-9-dihydroerythronolide B (**9**) and erythronolide B (**2**) were comparisons with model compounds used. The spectrum of the hemiketal form **3b** of 5-deoxy-5-oxoerythronolide B was weak because of low solubility. The resonance peak at 141.0 corresponding to A8 is expected by comparison with other derivatives of this type. The other peaks which were located were assigned by analogy.

For the methyl resonances, the only completely assigned set was for (9*S*)-9-dihydroerythronolide B (**9**). This was accomplished by SFD, using data from Demarco[7] and from the known effect of reducing a ketone on the adjacent carbons.[2a] In most cases, proton-shift data were unavailable, or the proton peaks were too close together to be useful. The A14 and 6-Me carbons were usually assignable from their chemical shift, while A15 was usually assigned by SFD, because its proton is generally the farthest upfield, and the carbon shift varied little. Some of the assignments of 12-Me were made by SFD, because the methyl protons generally appear at higher field than all the others, except A15. The remaining assignments of 12-Me were made assuming only small shift differences. An argument in favor is that this part of the ring appears to be conformationally invariant.[6] Furthermore, the carbon shifts for A12 already assigned were nearly constant except in 11-acetyl derivatives in

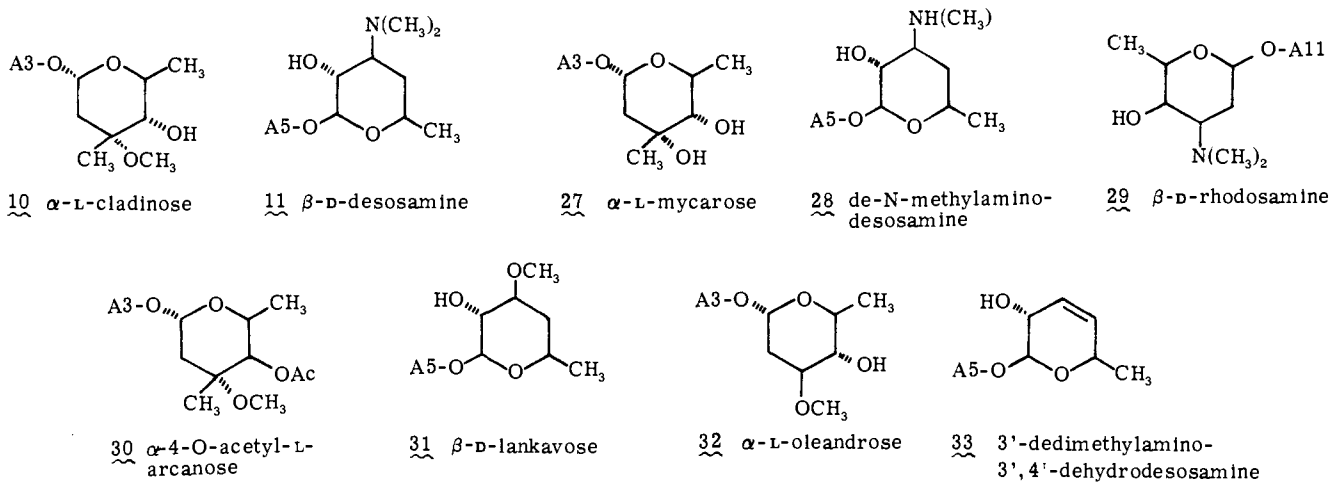


Figure 6.

which a 1.0–1.5 ppm upfield shift is observed. The corresponding shifts of 12-Me in these derivatives seem to be downfield. The remaining methyls fall into a reasonable pattern based on comparison with (9*S*)-9-dihydroerythronolide B and known effects. The two high-field methyls, in all but the 3,5-diacetyl derivatives, correspond to 4-Me and 8-Me. These are axial-like in the expected conformation. In particular, the 4-Me is held in a very unfavorable axial position because of the apparent hydrogen bonding between the hydroxyls on A3 and A5 (Figure 7a). It is significant that the chemical shifts of 4-Me in the nonsubstituted (at A3 and A5) derivatives are among the highest reported for any methyl in a hydrocarbon.[8] Differentiation between 4-Me and 8-Me is made by comparison of the various derivatives, and the most likely assignments are shown. The remaining methyls, 2-Me and 10-Me, are therefore downfield in the various derivatives. The only exceptions are in the 3,5-diacetyl compounds in which 2-Me is shifted upfield. The effect of acetoxy substitution on the γ carbons will be discussed more fully later. The differentiations of the 2-Me and the 10-Me are made by analogy, with the more likely assignment shown. Further support for these assignments will be given in the discussion of the erythromycin spectra. Most of the methyl peaks for the hemiketal of 5-deoxy-5-oxoerythronolide B were not found.

2. The Aminosugars. The ^{13}C NMR resonances for cladinose (**10**) and desosamine (**11**) are shown in Table IV. No SFD was done on the free sugars, but a number of assignments were made by comparison with gylcoside spectra which were verified by SFD. The peaks for the anomers were correlated by the procedure of Dorman and Roberts.[9] In the α anomers, the 1,3-diaxial interactions with the 3- and 5-carbons result in upfield shifts of carbons 1, 3, and 5. The shift is 5–6 ppm for D3 and D5 of desosamine and for C5 of cladinose. For C3, the shift was 1 ppm; however, this is a quaternary carbon. No sugars with similar quaternary carbons were studied by Dorman and Roberts.[9] The smaller upfield shift for the quaternary center may be due to the fact that steric compression is felt more strongly by a carbon with a directly bonded proton. Negative γ steric effects have been observed for quaternary carbons in alkanes.[8] Corresponding upfield shifts of C1, C2, D1, and D2 are also observed (comparing anomers).

3. Monoglycosides. Assignments for two monoglycosides, 3-*O*-α-L-mycarosylerythronolide B (**12**) and 5-*O*-β-D-desosaminylerythronolide B (**13**) are given in Table V. The sample of **13** was somewhat difficult to work with because of partial decomposition.

The assignments in the oxygenated region were made relatively easily by SFD. The pattern is the same as in the aglycon derivatives with the exception of the carbon carrying the sugar substituent. The assignments of the sugar peaks were also made by SFD.

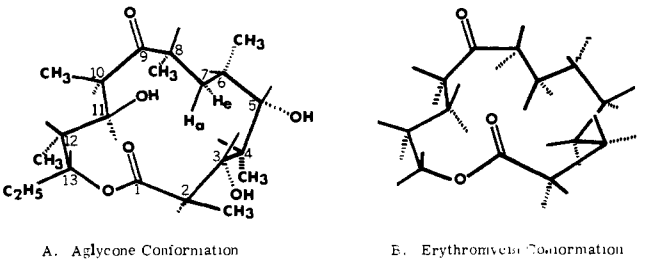


Figure 7. Proposed conformations (after Egan[6]).

The methine region was again the most difficult to assign. Assignments of **12** were made by SFD. However, it was here that the trouble with **13** was greatest. The assignments shown are the most likely and are consistent with repeated SFD experiments. The signals for A12, D-NMe_2, and A10 were often unresolved.

The methyl peaks were assigned by comparison with the aglycon spectra. These monoglycosides appear to be conformationally similar to the aglycon derivatives investigated by Egan.[6]

4. The Erythromycins. Included in this group are the parent antibiotics erythromycin A (**14**) and B (**15**) and several derivatives (**16–24**) made by acid degradation or modification of the glycosidated desosamine. The assignments are given in Tables VI–IX. Complete proton data[6,10] were available for all but the modified desosamine derivatives. 3'-Dedimethylamino-3',4'-dehydroerythromycin A (**18**) was only slightly soluble.

The oxygenated carbons were assigned by ORD and SFD where proton data were available, and by comparison otherwise. The only difficulty in this region was encountered from the fact that the quaternary carbons A6 and A12 are coincident in erythromycin A (**14**).

Except for the modified desosamine derivatives, assignments of the sugar peaks were straightforward. These assignments were independently verified by SFD. In the de-*N*-methyl derivatives, the assignments were made based on the expected substituent and steric effects. In such pyranose sugars, the effects tend to be reasonably predictable.[9] The downfield shift of D2 in going to the de-*N*-methyl derivatives is a negative γ effect, and the upfield shifts of D3 and D-NMe are negative β effects. That is, removal of the methyl group causes these shifts relative to the naturally oc-

Table IV. ^{13}C Shifts and Assignments for Cladinose and Desosamine (ppm from CS_2)

Compd	C1	C2	C3	C4	C5	C6	C3-OMe	C3-Me
α-Cladinose (10)	101.1	156.0	116.9	114.9	128.3	174.6	142.5	171.9
β-Cladinose	100.6	153.3	117.8	114.7	121.8	174.6	143.8	171.9

Compd	D1	D2	D3	D4	D5	D6	D-NMe_2
α-Desosamine	99.6	122.6	132.4	163.4	127.5	171.6	152.4
β-Desosamine (11)	94.4	120.8	127.5	163.4	123.5	171.6	152.1

Table V. Monoglycoside ^{13}C NMR Shifts and Assignments (ppm from CS_2)

	12	13		12	13		12	13		13		12
A9	−26.3	−22.1	A2	148.2	147.9.	A14	166.8	167.3	D1	86.4	M1	91.8
A1	17.4	14.5	A8	147.1	149.8	6-Me	167.2	165.1	D2	122.0	M2	151.4
A3	102.9	115.0	A10	153.6	152.7[d]	12-Me	183.7[c]	183.8	D3	127.1	M3	122.1
A5	110.9	99.6	A4	156.4	154.2[d]	4-Me	174.9[a]	185.7[b]	D4	164.7	M4	116.0
A6	117.3	117.8	A12	152.5	152.3	10-Me	177.0	176.9	D5	122.6	M5	125.7
A11	122.6	122.0	A7	155.8	151.6[d]	2-Me	183.7[c]	176.9	D6	171.7	M6	174.4[a]
A13	117.3	116.7	A15	182.4	182.3	8-Me	184.1[c]	185.3[b]	D-NMe_2	152.5	M3-Me	174.9[a]

[a-d] These assignments are interchangeable with others of the same superscript.

Table VI. Erythromycin ^{13}C NMR Shifts and Assignments for Oxygenated Aglycon Carbons (ppm from CS_2)

	14	15	16[a]	17[a]	18	19	20	21	22
A9	−29.4	−27.2	−28.2	−26.3	−29.1	76.4	72.7	41.4	41.4
A1	16.2	16.4	16.7	16.6	16.4	13.2	13.9	14.5	16.0
A3	112.3	112.1	112.4	112.1	112.2	116.5	121.9	115.5	110.5
A5	108.7	108.7	108.2	108.1	108.3	105.5	105.5	112.2	110.5
A6	117.5	117.5	117.8	117.7	117.3	110.3[b]	110.6	106.5	108.6
A11	123.5	123.1	123.6	123.2	123.4	106.5	64.0	121.3	121.9
A13	115.3	117.5	115.6	117.7	115.3	117.3	113.7	115.1	114.6
A12	117.5	153.2	117.8	153.1	117.3	111.0[b]	103.8	148.2	146.7

[a] CH_2Cl_2 as solvent. [b] Interchangeable assignments.

Table VII. Erythromycin ^{13}C NMR Shifts and Assignments for Sugar Carbons (ppm from CS_2)

	14	15	16[a]	17[a]	18	19	20	21
D1	89.1	89.4	89.7	89.7	89.6	89.5	88.0	89.8
D2	121.4	121.5	118.1	118.2	122.0[b]	123.0[d]	122.5	121.5
D3	126.8	126.9	132.4	132.3	60.2[c]	127.4	126.7	126.7
D4	163.6	163.6	160.1	160.0	66.1[c]	163.7	163.7	163.6
D5	123.5	123.6	124.0	124.0	123.4[b]	122.8	123.2	123.7
D6	171.0	171.2	171.6	171.4	171.0	171.2	171.0	171.5
D3-NMe_2	152.1	152.2	155.7	155.6		152.3	152.4	152.3
C1	96.0	95.9	96.1	95.9	95.8	97.8		98.0
C2	157.4	157.5	157.6	157.5	157.4	157.9		157.6
C3	119.7	119.9	119.9	119.9	119.7	119.7		119.3
C4	114.3	114.6	114.5	114.6	114.4	114.2		114.2
C5	126.8	126.9	127.0	126.9	126.8	126.7		126.7
C6	173.8	174.0	174.1	174.2	174.1	174.9		174.2
C3-OMe	142.9	143.1	143.2	143.3	143.0	143.4		142.9
C3-Me	171.0	171.2	171.3	171.4	171.0	171.6		171.0

[a] CH_2Cl_2 as solvent. [b-d] The assignments are interchangeable within the same vertical column of shifts having the same superscript.

Table VIII. Erythromycin ^{13}C NMR Shifts and Assignments for Methine Carbons (ppm from CS_2)

	14	15	16[a]	17[a]	18	19	20	21	22
A2	147.4	147.6	147.5	147.5	147.3	146.7	152.0[b]	147.9	148.2
A8	147.4	147.6	147.9	148.1	147.1	141.6	145.8	91.4	90.9
A10	154.3	153.2	154.1	153.1	153.8	151.0	53.4	158.4	158.7
A4	152.9	152.9	153.1	152.5	152.7	149.6	147.8	149.2	157.6
A12	117.5	153.2	117.8	153.1	117.3	111.0	103.8	148.2	146.7
A7	153.9	154.6	154.1	154.4	153.8	151.0	149.6[b]	150.1	150.3

[a] CH_2Cl_2 solvent. [b] Interchangeable assignments.

Table IX. Erythromycin ^{13}C NMR Shifts and Assignments of Methyl Carbons (ppm from CS_2)

	14	15	16[a]	17[a]	18	19	20	21	22
A14	171.0	166.8	171.3	166.9	171.0	168.9	168.9	166.4	166.7
6-Me	165.6	165.5	166.1	165.7	165.7	164.9	163.2	167.5	164.3
12-Me	174.0	183.3	174.3	183.6	174.1	167.8	169.2	183.8	184.3
4-Me	183.2	183.3	183.1	183.6	183.0	180.6[d]	179.9[e]	183.5	186.5
10-Me	176.4[b]	174.0	176.4[c]	174.2	176.4[b]	178.3	178.9	179.4[f]	177.5[b]
2-Me	176.1[b]	176.8	176.8[c]	177.0	176.1[b]	175.2[d]	179.0[e]	177.3[f]	178.6[b]
8-Me	180.3	183.3	180.7	183.0	180.6	179.2[d]	180.8	180.4	180.6
A15	181.7	182.2	182.1	182.4	181.8	181.9	182.5	182.1	182.3

[a] CH_2Cl_2 as solvent. [b-f] These assignments are interchangeable within the same vertical column of shifts among those having the same superscript.

Table X. Picromycin (23) ^{13}C NMR Shifts and Assignments (ppm from CS_2)

A3	−20.2	D2	122.7[a]	D4	164.2
A9	−10.5	D5	122.9[a]	12-Me	169.4
A1	+22.2	D3	126.7	A14	169.8
A11	47.0	A2	139.4	D6	171.6
A10	63.7	A8	145.7	2-Me	175.3[b]
D1	87.7	A4	149.6	6-Me	177.9[b]
A5	109.4	NMe_2	152.5	8-Me	178.0[b]
A13	111.0	A7	155.1	4-Me	179.2[b]
A12	117.7	A6	156.9	A15	182.1

[a,b] Interchangeable assignments.

Table XI. Lankamycin (24) ^{13}C NMR Shifts and Assignments (ppm from CS_2)

A9	−22.1	A13	121.6	L4	161.9
A1	+16.0	L2	123.8	8-Me	165.6
A11-Ac	22.2[a]	L5	125.3	11-AcMe	171.7
C4-Ac	22.8[a]	C5	130.0	C4-AcMe	171.7
L1	90.1	L3-OMe	135.7	L6	171.7
C1	95.8	C3-OMe	143.2	C3-Me	171.7
A5	108.1	A2	147.7	14-Me	173.2[c]
A8	112.3	A7	148.4	C6	173.7
A3	112.3	A14	150.1[b]	15-Me	175.7[c]
A15	114.9	A6	153.5[b]	2-Me	178.0
L3	117.0	A12	153.5	10-Me	181.6
C4	118.6	A10	154.4	4-Me	182.5
A11	119.4	C2	155.3	12-Me	182.5
C3	119.9	A4	158.4	6-Me	182.5

[a-c] Interchangeable assignments.

curring di-*N*-methyl derivatives. The upfield shift of D5 is a negative, remote steric effect.

The methine region was assigned primarily by SFD and ORD. Some of the difficulties encountered in the aglycon spectra were also encountered here. The problems caused by overlapping peaks were acute in this region; however, repeated SFD experiments allowed the assignments to be made independently of shift comparisons.

Overlapping of peaks is also acute in the methyl region. The proton shifts are available,[10] but many of the proton resonances are too close together to allow differentiation in the carbon spectra. One particularly critical assignment was the differentiation of 2-Me and 10-Me in erythromycin B (**15**). Here, the proton resonances were separated by 0.20 ppm, and the assignment could be made. The decision is fortified by the observed invariance of vicinal proton couplings in the A2–A5 region.[6]

5. Other Macrolides. Assignments for four other naturally occurring macrolides, picromycin (**23**), lankamycin (**24**), oleandomycin (**25**), and megalomicin A (**26**)[11] are given in Tables X–XIII. The picromycin and megalomicin A assignments were made from ND (noise-decoupled) and ORD spectra by comparison with the erythromycin spectra and other established ^{13}C NMR criteria. The oleandomycin and lankamycin assignments were made primarily from SFD spectra. The required proton shifts are available.[6]

Discussion

1. Substituent and Steric Effects. A number of regularities are discernible in the ^{13}C NMR spectra of the macrolides studied here which have root in commonly observed effects on carbon shifts. Several of the compounds differ by the presence or absence of hydroxyl groups at A6 or A12. Removing the hydroxyl group causes a 35–40 ppm upfield shift at the directly bonded carbon and a 9–10 ppm upfield shift at the *gem*-methyl, except for the 5-oxo derivatives. These are of the expected magnitudes. The effects at the other β carbons are considerably diminished. Removing the 6-hydroxyl causes a 3–5 ppm upfield shift of A5 (when hydroxylated) and A7. The effect on A5 as a ketone is only 1.2 ppm. Substituent effects on a ketone are expected to be

Table XII. Oleandomycin (25) ^{13}C NMR Shifts and Assignments (ppm from CS_2)

A9	−15.9	O5	123.2	A6	161.0
A1	+15.7	D3	127.0	D4	163.5
D1	87.7	A8	129.7	D6	171.2
O1	97.8	O3-OMe	136.0	13-Me	172.7[a]
A5	108.6	A8-CH_2O	143.4	O6	174.1[a]
A3	111.5	A2	147.7	2-Me	174.4[b]
O3	114.6	A10	147.7	6-Me	178.1[b]
O4	116.5	A12	148.6	10-Me	182.8[b,c]
D2	122.0	A7	150.7	12-Me	183.5[c]
A13	122.0	D-NMe_2	152.2	4-Me	185.1
D5	123.2	O2	158.4		
A11	123.2	A4	160.5		

[a-c] Interchangeable assignments.

Table XIII. Megalomicin A (26) ^{13}C NMR Shifts and Assignments (ppm from CS_2)

A9	−28.9	R4	123.2	D4	163.8
A1	16.9	D5	123.5	R2	164.4
D1	87.9	M5	125.1	6-Me	166.6
M1	93.9	D3	126.8	A14	170.9
R1	102.0	R3	126.8	D6	171.2
A5	107.3	R5	132.9	M3-Me	173.6
A3	109.4	A8	146.6	M6	173.8
A12	112.0	A2	147.6	12-Me	174.0
A13	115.5	R-NMe_2	149.9	10-Me	176.0
M4	115.7	A4	151.3	R6	176.0
A6	117.9	D-NMe_2	152.2	2-Me	177.4
A11	118.9	A7	153.6	8-Me	180.2
D2	121.2	A10	154.7	A15	181.9
M3	122.8	M2	155.2	4-Me	182.9

smaller.[2a] The effect of removing the 12-hydroxy is only +2 ppm at A13, almost nil at A11, and −4 ppm at A14. Smaller β effects are expected in more highly substituted molecules as discussed earlier, but it is likely that conformational changes associated with removing or adding these hydroxy groups contribute also. These changes will be discussed later.

The effects of going from a hydroxyl to a ketone and the reverse appear to be either larger or comparable to the magnitudes observed for simpler systems. Reducing the ketone at A9 shifts the β methines upfield 7–8 ppm and the γ-methyls downfield 1–2 ppm. The β effect here is comparable to that observed in cyclohexane systems,[2a] while γ effect is smaller. The effect of oxidizing A5 to a ketone is 4–5 ppm downfield at A6 when hydroxylated and 10 ppm downfield in the 6-deoxy derivatives. The β effect at A4 is 4 ppm downfield. The effects at the γ-methyls are variable. Conformational changes appear to be important here, because the flexible part of the ring (A6–A9) is affected.[6]

The effects of acetoxy and methoxy groups relative to the free alcohols have been studied in several systems.[12] The general trend for acetoxy substitution seems to be a downfield shift at the α carbon, an upfield shift at the β carbon, and little or no effect elsewhere. When A11 is acetylated, there is a 2–3 ppm downfield shift at A11, a 1–1.5 ppm upfield shift at A12, and a 1 ppm upfield shift at A13. These are reasonable based on the earlier work. The effects at A9 and A10 show less conformity. The inconsistencies (as compared with simple models) are almost certainly caused by the conformational changes which accompany 11-acetylation and which will be discussed more fully later. It is worthwhile to note that the A10–A13 region does not change throughout the erythromycin B series as indicated by the constant vicinal proton–proton couplings.[6] The success of the simple model in predicting the acetylation shifts at A11–A13 is consistent with this conclusion.

The effect of acetylation at A3 and A5 is complicated by possible conformational changes of the carbons. Egan[6] has shown that the conformations of the 3,5-diacetyl derivatives

resemble the conformations of the natural antibiotics in which the substituent at A3 moves above the plane of the ring, and the substituent at A5 moves down (Figure 7). In the 3,5-diacetyl derivatives, the shifts of A3 and A5 are *upfield* 1–4 ppm. There are also upfield shifts of 1–2 ppm at A2, A4, and A6. The effects at the γ carbons are more profound. A1 is shifted upfield 1–2 ppm, 2-Me is shifted upfield 5–6 ppm, 4-Me is shifted downfield 3–4 ppm, 6-Me is essentially unchanged, and A7 is shifted upfield 3–4 ppm.

As noted earlier, acetylation generally has little or no effect at γ carbons so an explanation is in order. The fact is that these shifts are quite consistent with expected conformational changes arising from the free hydroxyls in the nonacetylated derivatives being diaxial and hydrogen bonded. Since no hydrogen bonding is possible in the diacetyl derivatives, the acetyl groups are more free to rotate, and the steric effects of this substituent are felt at the γ carbons. The upfield shifts at 2-Me and A7 are consistent with the nearest acetyl group moving closer as in the proposed conformation. The downfield shift at 4-Me is a result of the relief of 1,3-diaxial steric interactions in the nonacetylated derivatives. As shown in Figure 7a, the 4-Me group is held in an unfavorable axial position by the hydrogen-bonded hydroxyl groups. Figure 7b shows how the 4-Me can move away from the center of the ring. This conclusion is consistent with the assignments given earlier.

The effect of methyl substitution at oxygen and nitrogen can be assessed by comparing mycarose **27** with cladinose **10** and the de-*N*-methyl derivatives with desosamine **11**, respectively. Mycarose differs from cladinose by methylation of the 3-hydroxyl group (see Tables IV, V, and VII). O-Methylation of axial hydroxyls of inositols has been shown to cause downfield shifts at the α and β carbons and little or no effect elsewhere.[13] The only significant deviation from this pattern is at carbon-5 where an upfield shift is observed in going from mycarosyl to either cladinose or cladinosyl. The change is only +1.2 ppm in the glycosidated sugars, however.

Most of the steric effects observed are associated with the conformational changes to be discussed more later. One change that is simply explained is the upfield shift at A2 in the nonglycosidated derivatives. It has already been pointed out that 4-Me is forced down by the hydrogen bonding in these derivatives. This increases the 1,3-diaxial interaction between A2 and A4 and causes the observed upfield shift.

It is generally accepted that carbons bearing axial hydroxyl groups appear upfield from those with equatorial hydroxyl groups in cyclohexane derivatives.[14] These considerations seem to be only partially applicable to this study. The hydroxyls at A11 are always axial, and this carbon comes at higher field (121–123 ppm) than the other carbons carrying hydroxyls. The hydroxyls on A3 and A5 are always equatorial, and these carbons are at low fields in unmodified derivatives (111–115 ppm). However, A6 which is primarily axial and A12 which is equatorial when hydroxylated are coincident in erythromycin A. Worse yet, A9 in 9*S*-9-dihydroerythronolide B has been shown to be axial[15] yet appears at 110.9 ppm. A possible explanation is that this position is on a "corner" of the ring in the proposed conformation and then is free of diaxial interaction except with the hydroxyl at A11. Differentiation of axial and equatorial methyls by their chemical shifts is much clearer. The axial methyls 4-Me, 8-Me, and 12-Me are always upfield from the equatorial methyls 2-Me, 6-Me, and 10-Me. The average difference is about 6 ppm. A priori use of these simplified considerations on molecules of this complexity is probably not justified; nonetheless, reasonable agreement is obtained.

2. Hydrogen Bonding. It appears that ^{13}C NMR is very useful in assessing intramolecular hydrogen bonding in macrolides. A downfield shift of carbonyl carbons which have their oxygens involved in hydrogen bonding has been reported.[16] Examination of the ketone shifts in the various derivatives studied here shows a variation of about 9 ppm. The resonance of A9 for erythromycin A **14** is −29.4 ppm and is one of the most deshielded organic carbonyls reported so far. By comparison, the diisopropyl ketone carbonyl carbon peak appears at −22.8 ppm.[17] Because it is unlikely that ring strain (which can cause a downfield shift)[18] is much of a problem here, and because steric effects should cause an upfield shift, intramolecular hydrogen bonding provides the most reasonable explanation of these unusual shifts. Examination of the proposed conformations (Figure 7) reveals that hydrogen bonding to the oxygen of A9 could be from either the hydroxyl group at A11 or A6. It appears, however, that the dominant bonding is from hydroxyls at A6. The argument is that erythromycin A (**14**) and megalomicin A (**26**) (which has a sugar substituent at A11) show ketone shifts of −29.4 and −28.9 ppm, respectively. In all 6-deoxy derivatives, the ketone carbon resonance comes at −20.7 to −23.0 ppm. For lankamycin (**24**) which lacks a 6-hydroxy group and has an 11-acetoxy group (and also an 8-hydroxyl), the ketone carbon is at −21.9 ppm. These latter values are comparable to what would be expected for an unstrained tetrasubstituted ketone. Furthermore, 11-acetylation causes a 1–2 ppm upfield shift of the ketone in the 6-deoxy derivative which could be attributed to loss of hydrogen bonding and/or steric effect.

The shift data also suggest that there is hydrogen bonding between the A11 hydroxy and the A1 lactone carbonyl. In all derivatives with an A11 hydroxy substituent (**5, 6, 8, 26**), A1 appears upfield compared with the corresponding substituent-free (at A11) derivatives. While this could conceivably be a steric effect in the acetoxy derivatives (**5, 6, 8**), this seems unlikely in megalomicin A (**26**). The possibility of hydrogen bonding between the A11 hydroxy and the A1 carbonyl may also explain the apparent dominance of hydrogen bonding from the A6 hydroxy to the A9 ketone. Other differences in the observed shifts of A1 will be discussed later.

We have noted that the carbons bearing the sugar substituents in the monoglycosides appear at very low field (Table V). This is also most likely a result of hydrogen bonding from the corresponding free hydroxyl; that is, the hydroxyl at A3 is hydrogen bonded to the glycosidated oxygen at A5 in 5-*O*-β-D-desosaminylerythronolide B (**13**). In the 5-oxo derivatives, the A5 ketone carbon again appears at low field (see Table I) probably because of hydrogen bonding from the hydroxyl on A3. It has been determined that these derivatives (5-oxo) are conformationally similar to the others in the A2–A5 region.[19]

Also significant is the 8-ppm *upfield* shift of A3 in the 5-oxo derivatives (Table I). Available evidence indicates a *downfield* shift is expected for the hydrogen donor in hydrogen bonding situations. In particular, the carbon of chloroform was found to shift downfield in oxygenated solvents.[20] Downfield shifts are also observed in hydrogen bonding with phenolic hydroxyls such as in salicylaldehyde and salicylic acid derivatives.[21] For a further check on this, a ^{13}C NMR spectrum of 4-hydroxy-4-methylpentanone was taken. The shifts for this material and some other alcohols are shown in Table XIV. At most, a 1-ppm upfield shift is observed at the hydroxyl carbon of the ketone. For this reason, the upfield shift of A3 in the 5-oxo derivatives relative to 5-hydroxyl derivatives is most probably caused by the lack of hydrogen bonding to the hydroxyl at A3 and an apparent conformational change at the lactone. A1 is shifted upfield in these derivatives also.

3. Conformational Considerations. The ^{13}C NMR shift

Table XIV. Comparison of Some ^{13}C NMR Shifts for Alcohols (ppm from CS_2)

	C1	C2	C3	C4	C5	Me
4-Hydroxy-4-methyl-2-pentanone	161.0	−16.7	137.8	123.3	163.2	163.2
2-Butanol[a]	169.9	123.8	160.5	182.6		
2-Methyl-2-butanol[a]	163.9	122.2	156.0	184.0		163.9
2-Pentanol[a]	169.2	125.5	150.9	173.4	178.5	

[a] Data from ref 2a, p 141.

data described here are consistent with several subtle conformational differences among the various derivatives. For example, the spectra for erythromycin A (**14**) and erythromycin B (**15**) show a number of differences that have not already been accounted for by simpler considerations. In particular, the changes at A9, A10, 10-Me, and 8-Me are significant. The change from **14** to **15** (removal of the 12-hydroxy group) causes changes upfield at A9 of 2.2 ppm, at A10 downfield of 1.1 ppm, at 10-Me downfield of 2.1–2.4 ppm, and at 8-Me upfield of 3.0 ppm. However, the ^1H NMR spectra for **14** and **15** indicate no significant differences in any of the vicinal proton-proton couplings on the ring.[6] Furthermore, it was demonstrated earlier that the dominant interaction at the A9 ketone is likely to be hydrogen bonding with the hydroxyl group at A6. These results taken together are consistent with the following conformational differences between **14** and **15.** In erythromycin A (**14**), the 10-methyl moves down to avoid the 1,3-diaxial interaction with the 12-hydroxyl by a rotation about the 9,10 bond. This moves the ketone into the ring closer to the 6-hydroxyl. There is no change in the 10,11 bond but a slight rotation around the 11,12 bond (Figure 8). Further evidence for this change can be obtained from circular dichroism data for these two ketones as determined by Mitscher and Slater.[22] The effect of this change is to drive the 10-Me into a positive quadrant of the ketone in erythromycin A. The observed rotations are −6600° for erythromycin A and −10800° for erythromycin B. Because the 12-hydroxy is in a negative quadrant, its removal should cause an increase rather than a decrease in the observed rotations going from erythromycin A to B (Figure 8). This change is also consistent with the ^{13}C NMR data. The A9 ketone is closer to the 6-hydroxyl in erythromycin A (**14**) and hence can be more strongly hydrogen bonded, which explains the observed downfield shift. The 10-Me and A10 shifts move downfield as a result of reduced steric compression. The 8-Me moves upfield because of increased diaxial interaction with A10 across the ketone. Alternatively, a rotation around the 8,9 bond could also occur. Because both the ^1H NMR and ^{13}C NMR spectra indicate no significant differences between these two erythromycins anywhere else, the nature of this change could be important in explaining the differences in antibiotic activities.

Conformational differences could also be responsible for the bewildering scatter of the methine peaks in the aglycon derivatives. Egan[6] has considered this problem with the aid of the observed differences in vicinal proton-proton couplings. The differences between the proton spectra of erythronolide B (**15**) and 11-acetylerythronolide B (**5**) were explained by assuming that the two conformers shown in Figure 9 are populated differently in the two derivatives. In particular, 11-acetylerythronolide B (**5**) was assumed to exist in conformer B more than the other derivatives.[6] The relevant carbon shifts offer evidence in favor of this argument. Carbon A7 in 11-acetylerythronolide B (**5**) appears at 150.6 ppm, shifted considerably downfield from the other aglycon derivatives (Table II). A similar downfield shift of A7 is observed in the spectra of anhydroerythromycin A (**19**) and erythralosamine (**20**) relative to erythromycin A,

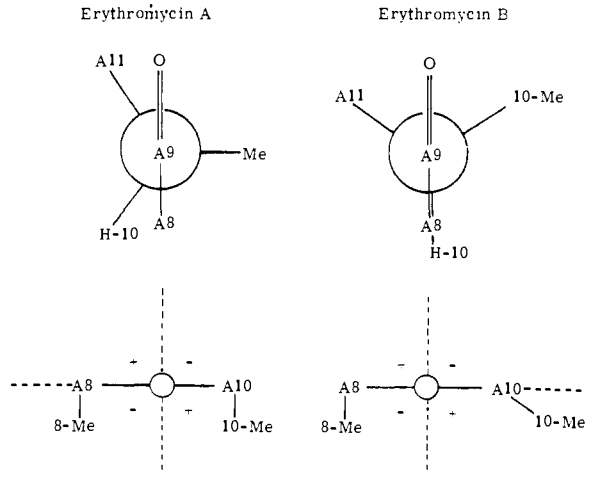


Figure 8. Conformational differences between erythromycin A and B.

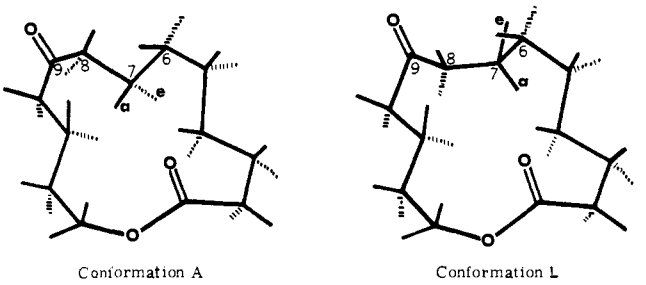


Figure 9. Possible equilibrating aglycon conformations (after Egan[6]).

and in the spectrum of the hemiketal of 5-deoxy-5-oxoerythronolide B relative to the ketoform. The carbons in the furanose form of fructose appear downfield from those in the pyranose form.[23] The proposed conformation for 11-acetylerythronolide B (**5**) has this region of the ring in a pseudo-five-membered ring, and the analogy to the hemiketals has also been made, by Egan.[6] Furthermore, this conformation has the A6 hydroxy group directed into the ring and poorly oriented for hydrogen bonding with the ketone at A9. The observed upfield shift of A9 in 11-acetylerythronolide B (**5**) offers further evidence for this conformation.

It appears that the spectra of the other substances which could be conformationally mobile partially support this contention. Table XV gives relevant data for several derivatives which differ only by their oxygen substituents. The strongest correlations among the first six examples are between A8, A9, H-8, and the vicinal couplings. The proposed conformational equilibration[6] requires **15, 12,** and **7** to prefer conformation A (Figure 9) and **11, 8,** and **24** to be mixtures of conformations A and B. This is based on the observed proton chemical shifts of H-8 and the vicinal couplings.

A grouping of the shifts for A8, A9, H-8, the vicinal couplings, and A10 is apparent, although the shifts of A10 are evenly distributed over a range of 1.5 ppm. This general trend for A7 on the remaining derivatives is not apparent; however, it appears that the shift of A7 is most sensitive to the substitution at A5. A pattern for 8-Me is not evident. The temperature dependence of these shifts was not investigated as was done in the earlier study.[6]

A number of changes other than those already discussed occur in the 6-deoxy derivatives. Relative to the 6-oxygenated derivatives, 6-deoxyerythronolide B (**1**) and 11-acetyl-6-deoxyerythronolide B (**6**) have A8 upfield 3 ppm and A10 downfield 4–5 ppm (Table III). The upfield shift of A8 is most probably caused by an increased 1,3-diaxial interaction of its proton with 6-Me. This is consistent with the proposed conformation and observed vicinal couplings. The

Table XV. Comparison of ^{13}C and ^{1}H NMR Parameters for Several Aglycon Derivatives

	A8	A7	A9	A10	8-Me	H-8[a]	$J_{7a,8}$,[a,b]	$J_{7e,8}$,[a,b]
15	147.6	154.3	−26.4	153.2	183.6	2.76	10	3
12	147.1	151.4	−26.3	153.6	183.5–184	2.70	9	4
7	147.1	155.0	−27.2	154.0	183–183.5	2.74	9	3
11	150.6	151.6		153.1	185.0		7	7
8	150.3	155.8	−22.5	152.5	182.4–183.7	2.88		
24	149.8	151.6	−22.1	152.7	185.3	2.80	7	8
5	149.9	150.6	−20.5 ± 1	153.4	184.8	3.18	3	12

[a] From ref 6. [b] Approximate values in hertz.[6]

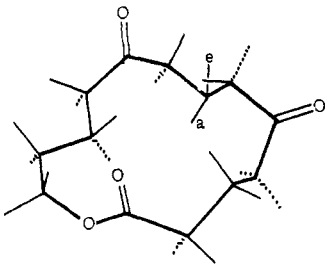


Figure 10. Proposed conformation for 5,6-dideoxy-5-oxoerythronolide B (**4**).

Table XVI. Some Comparative ^{13}C and ^{1}H NMR Data for 5,6-Dideoxy-5-oxoerythronolide B (4) and 6-Deoxyerythronolide B (1)

	A8	A10	$J_{6,7a}$	$J_{6,7e}$	$J_{7a,8}$	$J_{7e,8}$
4[a]	145.9	153.4	8.2	8.2	3.0	13.0
1[b]	152.8	148.6	4.7	10.2	13.0	4.0

[a] Proton coupling in hertz from ref 19. [b] Proton coupling in hertz from ref 6.

reason for the downfield shift of A10 is not apparent but probably involves a change in the diaxial interaction across the ketone A9, because no changes are observed in the A11–A13 region in either the proton or carbon spectra. The change in going to the 5-oxo derivatives (Table III) is amazingly large. A8 moves downfield 7 ppm, and A10 moves upfield 5 ppm. Figure 10 shows a conformation compatible with the data in Table XVI. Here, A6, A7, and A8 are eclipsed carbons as in a rigid cyclopentane with the A6 proton pointing into the ring. This conformation resembles that expected for the hemiketal of 5-deoxy-5-oxoerythronolide B (**3b**). The reason for observed changes in the carbon spectra are now apparent. The 1,3-diaxial interaction between 6-methyl and the 8-proton has been relieved, while the interaction between the 8-methyl and 10-hydrogen has been increased. This could cause the observed shifts. The proposed conformation is also consistent with the observed vicinal 6–7 and 7–8 proton–proton couplings.[6,19]

Further conformational changes going from the 5-hydroxy derivatives to the 5-oxo derivatives are suggested by examining the significant ^{13}C NMR shifts of 4-Me, A3, and A1. In particular, the 4-Me is shifted downfield 3–4 ppm, A3 is shifted upfield 7–8 ppm, and A1 shifted upfield 2–3 ppm in the 5-oxo derivatives, relative to the analogous 5-hydroxy derivatives (Tables I–III). It has already been pointed out that the position of A1 is sensitive to substitution at the A11 hydroxyl because of hydrogen bonding and/or steric compression. However, it is apparent that substitution at the A3 hydroxy also causes an upfield shift of A1 (Tables I, V, and VI) in the nondehydrated derivatives. This is reasonable because the A3 oxygen is known to be tilted up in these compounds (Figure 7).[6] Because $J_{2,3}$ is nearly constant in all but erythromycins A and B, a rotation around the 1,2 bond must occur, forcing the lactone carbonyl into the ring (Figure 7). This causes an upfield shift of both A1 and A3 by steric interaction. That a substituent change may ultimately manifest itself as a conformational change at the relatively unhindered lactone is not too surprising in these heavily substituted compounds. A downfield shift of 4-Me is also observed in these derivatives with an A3 hydroxyl substituent (Tables III, V, and IX) because now the methyl is no longer held in as unfavorable an axial position as when A3 is tilted up (Figure 7). This is the same shift pattern that is seen in the 5-oxo derivatives relative to the 5-hydroxy derivatives. All of this is consistent with a conformation for the 5-oxo derivatives in which the A3 hydroxy is tilted up and hydrogen bonded to the A5 ketone oxygen. The A5 ketone group can likewise move up out of the plane of the macrolide ring since this motion is no longer hindered by a proton at A5. All these changes are depicted in the conformation of 5,6-dideoxy-5-oxoerythronolide B (**4**) shown in Figure 10. The observed downfield shifts of 4-Me and A1 are accounted for by analogy with the just-discussed A3 hydroxyl substituted cases. The upfield shift of A3 relative to the 5-hydroxy derivatives is caused by the *lack* of hydrogen bonding from A5 and the steric congestion around the A3 proton since it now points more into the ring (Figure 10). The constancy of the vicinal $J_{2,3}$ coupling constants and the ^{13}C NMR shift of A2 in the 5-oxo derivatives, the other aglycons, and the monoglycosides indicates A2 is also tilted up, keeping its relative position with A3 (cf. Figure 10). Furthermore, A2 is shifted downfield and $J_{2,3}$ reduced in erythromycins A and B[6,15,19] (see also Table VIII). The driving force for this change is probably the relief of the steric interaction at the 4-methyl which is held in an unfavorable axial position in the 5-hydroxy derivatives. Further evidence for this contention can be cited by comparing the expected *substituent* effect at a methyl adjacent to a hydroxy or a ketone group. In 2-methylcyclohexanone, the methyl appears at 179.0 ppm compared with 173.7 ppm in *trans*-2-methyl- and 176.3 ppm in *cis*-2-methylcyclohexanol.[2a] Thus, the expected substituent shift is upfield 3–6 ppm at the 4-methyl. In fact, a 2–3 ppm upfield shift is observed at 8-Me and 10-Me going from (9*S*)-9-dihydroerythronolide B (**9**) to the 9-keto derivatives, such as **2** (see Table III). The observed downfield shift of 4-Me in the 5-oxo derivatives is thus more noteworthy.

One conformational problem that has received little attention is the orientation of the 6-deoxy sugars with respect to the aglycon ring. The ^{13}C NMR results appear to shed some light on this. In the crystal structure of erythromycin A (**14**),[24] the sugars are roughly perpendicular to the ring with the 6-methyl groups above the plane of the ring.

A comparison of the free and glycosidated chemical shifts of the sugars is given in Table XVII. Glycosidation of the β anomer of desosamine (**11**) has little effect as might be predicted.[23] Glycosidation of the α anomer of cladinose has a greater effect, again as would be predicted. The interesting change here is the 2.8 ppm *upfield* shift of C3 on glycosidation. The resonance of a 3-carbon on an α-hexose would be expected to move downfield after glycosidation with a nonfreely rotating group because of a partial relief of

Table XVII. Comparison of the ^{13}C NMR Shifts Free and Glycosidated Sugars (ppm from CS_2)

	C1	C2	C3	C4	C5	C6	C3-Me	C3-OMe
α-Cladinose (10)	101.1	156.0	116.9	114.9	128.3	174.6	171.9	142.5
α-Cladinosyl[a]	96.0	157.3	119.7	114.4	127.0	174.1	171.8	143.1
	D1	D2	D3	D4	D5	D6	D3-NMe_2	
β-Desosamine (11)	94.4	120.8	127.5	163.4	123.5	171.6	152.1	
β-Desosaminyl[a]	89.6	121.5	126.8	163.9	123.5	171.6	152.1	

[a] Average values.

the 1,3-diaxial interaction.[9,23] In the crystal, the C3-OMe group is closest to the desosamine moiety and is the only group that seems to have a significant interaction with anything else in the molecule. The C3-OMe resonance also moves upfield, but only slightly, on glycosidation. The expected change again would be a downfield one. All of this is consistent with a conformation in solution similar to that in the solid state so that the observed upfield shifts at C3 and C3-OMe are caused by the remote (through many bonds) steric compressions.

Egan[6] has briefly mentioned that the proton shifts of the methoxy group on cladinose **10** are sensitive to substitution at the 3-position of desosamine (**11**). Similar effects might also be expected in the ^{13}C NMR spectra. However, the resonance of C3-OMe is found at 142.9, 143.2, and 143.0 in erythromycin A (**14**), de-*N*-methylaminoerythromycin A (**16**) (in CH_2Cl_2), and 3'-dedimethylamino-3',4'-dehydroerythromycin A (**18**), respectively. The differences between these shifts are clearly too small to be significant. The reverse comparison is no more significant. Megalomicin A (**26**) has a mycarosyl (**27**) substituent at A3 and desosaminyl (**11**) at A5. Mycarose (**27**) differs from cladinose (**10**) by the lack of the 3-*O*-methyl group. The shift of D3-NMe_2 in megalomicin A (**26**) is 152.2 compared with 152.1 for erythromycin A (**14**).

Further comparison of the de-*N*-methyl spectra with the natural antibiotics does yield interesting results. Neglecting effects already discussed on the desosamine shifts and changes which appear to result from overlapping peaks, a number of small but significant differences are apparent (Table XVIII). The effect at A5 could be dismissed as a remote substituent or steric effect but the others require an explanation. Working with models of the proposed conformation (Figure 7) indicates that it is possible for the desosamine grouping to move around to the side of the aglycon ring by rotation principally around the A5,O bond. This places the D-NMe_2 group near the 8-Me and the D2 proton near A7. Because most of the changes occur in the A7–A10 region, this seems to be a viable explanation for the observed changes on going to the de-*N*-methyl derivatives. Further evidence for the existence of this freedom for desosamine can be cited. Comparison of the desosamine peaks in the various anhydro derivatives (Tables VII) shows significant variations in the desosamine (**11**) peaks, particularly D2 and D3. Meanwhile, almost no changes are observed in the cladinose (**10**) spectra. Furthermore, A7 is upfield in erythromycin A (**14**) and B (**15**) relative to the unsubstituted (at A5) aglycons by about 3 ppm. It has already been observed that acetylation at A5 causes an upfield shift at A7. Thus the observed upfield shifts of A7 are consistent with steric interactions that would be caused by movement of the desosamine ring. An alternative explanation for the upfield shift of A7 on going from the aglycon conformation (Figure 7) is not apparent. In fact, A4 and 4-Me move away from A7 in the erythromycin conformation which would imply an expected downfield shift of A7. All of this evidence seems consistent with the cladinose group having a fixed conformation in solution similar to that in the crystal, while desosamine can move in the manner described.

Table XVIII. Comparison of ^{13}C NMR Shifts for Several Modified Desosamine Derivatives (ppm from CS_2)

	A9	A5	A3	4-Me	A8			
14[a]	−29.0	109.2	112.7	183.6	147.6			
16[a]	−28.2	108.2	112.4	183.1	147.9			
	A9	A5	A3	4-Me	A8	8-Me	A7	A10
15[b]	−27.2	108.7	112.1	183.3	147.6	183.3	154.6	153.3
17[b]	−27.1	107.4	111.9	183.1	147.8	183.1	154.0	152.9
	A9	A5	A8	A10	8-Me	D1		
14[b]	−29.4	108.7	147.4	154.3	180.3	89.1		
18[b]	−29.1	108.3	147.1	153.8	180.6	89.6		

[a] CH_2Cl_2 as solvent. [b] $CDCl_3$ as solvent.

A number of discernible, regular effects are apparent in the spectra of the various dehydro and ketal derivatives (**3b, 19–23**). It is here that the value of ^{13}C NMR in the structural analysis of macrolides is most apparent, because these kinds of derivatives play an important role in macrolide chemistry. Significant downfield shifts of carbons which end up in five-membered (furan) rings are observed (Tables II and VIII). The downfield shifts of A8 and A7 seem characteristic for the formation of the hemiketal and the saturated spiroketal. This effect was also observed by Dorman and Roberts[23] in a study of the ^{13}C NMR of the furanose and pyranose forms of fructose.

Going from erythromycin A (**14**) to anhydroerythromycin A (**19**), results in a 17-ppm downfield shift of A11. While this may be only due to the five-membered ring formation, it is possible that this shift is indicative of steric relief at A11 through loss of water. As such, this may be part of the driving force for dehydration.

Comparison of the ^{13}C NMR spectra for 8,9-anhydroerythromycin B-6,9-hemiketal (**21**) and 8,9-anhydroerythronolide B-6,9-hemiketal (**22**) show rather substantial upfield shifts of A4 and 4-Me on deglycosidation (Table IX). The shifts observed for **21** are the more typical for these compounds (Table IX). The assignments were confirmed by repeated SFD experiments. An explanation for the high-field shifts in **22** is apparent through examination of models. Hydrogen bonding between A3 and A5 forces the 4-Me down as in the other aglycon derivatives (Table III) causing the high-field shift of 4-Me. This causes a severe interaction between the A4 proton and the oxygen of the furan ring which accounts for the large upfield shift of A4.

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Pseudochirality[1,2]

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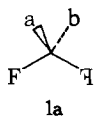
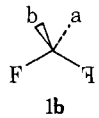
Abstract: An algebraic description of pseudochirality (pseudoasymmetry) is presented which is analogous to that given of regular chirality by Wheland. It is found that a chemical structure is pseudochiral if it lacks certain kinds of symmetry based on combinations of chirality reversal operations and point group operations. Pseudochirality groups are defined which include these operations. The derivation of these groups for chemical structures with one or more sets of chiral ligands is described, and a tabulation of general classes of pseudochirality groups based on the usual classes of point groups is given.

The concept of pseudoasymmetry is reasonably well known as a special case of stereoisomerism.[4] It is well known that carbon atoms with four different substituents are chiral, and that the enantiomeric forms can be interconverted by reflection in any mirror plane. However, if two of the substituents are themselves enantiomeric chiral ligands, two isomeric forms result which are meso and cannot be interconverted by reflection in a mirror plane. Such a pair of stereoisomers have been termed a *pseudoasymmetric pair*.[5] A symbolic example of such a pair is **1a** and **1b**, a real example is *meso*-pentaric acid. These are diastereomers and differ in physical properties such as melting point. In all chemical structures in this paper, chiral ligands are designated by open circles or by letters which are themselves chiral in two dimensions. The enantiomeric ligands are then designated by filled circles or reversed letters. This notation has been used extensively by Prelog.[6-9]

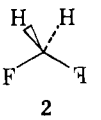


1a



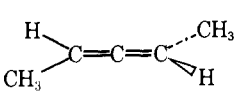
1b

An important distinction must be made between pseudoasymmetric structures (**1a** and **1b**) typically termed "meso" and structures such as **2** which exist in only *one* achiral meso form. Such a structure will be designated meso from now on, while the pseudoasymmetric structures will be termed pseudoasymmetric or pseudochiral. This is a departure from standard terminology which designates all such structures as meso. The intrinsic difference between the pseudochiral and meso situations will be established later in this paper.

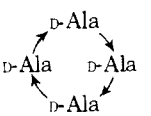


2

The purpose here is to give an algebraic description of this phenomenon analogous to that for regular chirality. A rigid structure is chiral if it lacks an alternating axis of symmetry.[10] It may, however, have pure rotation symmetry, such as 1,2-dimethylallene (C_2 symmetry) or cyclotetra-D-alanine (in a C_4 conformation).



C_2



C_4